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(71) Applicants (for all designated States except US):
RIBOZYME PHARMACEUTICALS, INCORPORATED [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US). CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608-2916 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): ESCOBEDO, Jaime [US/US]; 1470 Livorna Road, Alamo, CA 94507

(US). MCSWIGGEN, James [US/US]; 4866 Franklin Drive, Boulder, CO 80301 (US). PAVCO, Pamela [US/US]; 705 Barberry Circle, Lafayette, CO 80026 (US). STINCHCOMB, Dan [US/US]; 8409 South Country Road 3, Ft. Collins, CO 80528 (US). SANDBERG, Jennifer [US/US]; 620 Bluegrass Drive, Longmont, CO 80503 (US). GORDON, Gilad [US/US]; 3605 Silver Plume Lane, Boulder, CO 80303 (US).

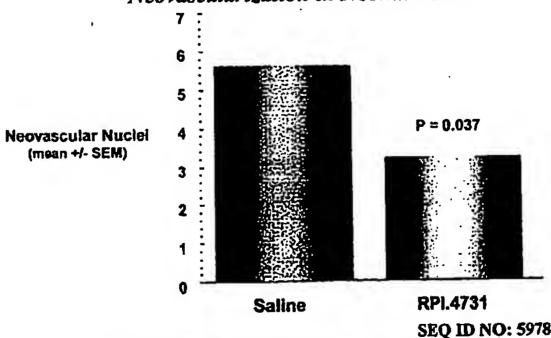
(74) Agent: TERPSTRA, Anita, J.; McDonnell Boehnen Hulbert & Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

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(54) Title: NUCLEIC ACID BASED MODULATION OF FEMALE REPRODUCTIVE DISEASES AND CONDITIONS

RPI.4731 Reduces Hypoxia-Induced Retinal Neovascularization in Neonatal Mice



Results: -40% decrease in retinal neovascularization following two intraocular injections of RPI.4731

(57) Abstract: The present invention relates to nucleic acid molecules, including dsRNA, siRNA, antisense, 2,5-A chimeras, aptamers, and enzymatic nucleic acid molecules, such as hammerhead ribozymes, DNAzymes, and allozymes, which modulate the expression of vascular endothelial growth factor receptor (VEGF) and/or vascular endothelial growth factor receptor (VEGF) genes for the treatment and/or diagnosis of diseases and conditions associated with angiogenesis, such as cancer, tumor angiogenesis, or ocular indications such as diabetic retinopathy, or age related macular degeneration, proliferative diabetic retinopathy, hypoxia-induced angiogenesis, rheumatoid arthritis, psoriasis, wound healing, and female reproductive disorders and conditions, including but not limited to endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), and menopausal dysfunction.

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NUCLEIC ACID BASED MODULATION OF FEMALE REPRODUCTIVE DISEASES AND CONDITIONS

This patent application claims priority from Sandberg et al., USSN 60/334,461, filed November 30, 2001, entitled "Method and Reagent for the Modulation of Female Reproductive Diseases and Conditions" and Pavco et al., USSN 10/138,674, filed May 3, 2002, which is a continuation in part of Pavco et al., USSN 09/870,161, which is a continuation-in-part of Pavco et al., USSN 09/708,690, filed November 7, 2000, which is a continuation-in-part of Pavco et al., USSN 09/371,722, filed August 10, 1999, which is a continuation-in-part of Pavco et al., USSN 08/584,040, filed January 11, 1996, which claims the benefit of Pavco et al., USSN 60/005,974, filed on October 26, 1995; these earlier applications are entitled "Method and Reagent for Treatment of Diseases or Conditions Related to Levels of Vascular Endothelial Growth Factor Receptor". Each of these applications is hereby incorporated by reference herein in it's entirety including the drawings and tables.

Technical Field Of The Invention

This invention relates to methods and reagents for the treatment of diseases or conditions relating to the levels of expression of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor(s). Specifically, the instant invention features nucleic-acid based molecules and methods that modulate the expression of vascular endothelial growth factor and/or vascular endothelial growth factor receptors, such as VEGFR1 and/or VEGFR2, that are useful in preventing, treating, controlling and/or diagnosing disorders and conditions related to angiogenesis, including but not limited to cancer, tumor angiogenesis, or ocular indications such as diabetic retinopathy, or age related macular degeneration, proliferative diabetic retinopathy, hypoxia-induced angiogenesis, rheumatoid arthritis, psoriasis, wound healing, endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), and menopausal dysfunction.

Background Of The Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

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VEGF, also referred to as vascular permeability factor (VPF) and vasculotropin, is a potent and highly specific mitogen of vascular endothelial cells (for a review see Ferrara, 1993 Trends Cardiovas. Med. 3, 244; Neufeld et al., 1994, Prog. Growth Factor Res. 5, 89). VEGF-induced neovascularization is implicated in various pathological conditions such as tumor angiogenesis, or ocular indications such as diabetic retinopathy, or age related macular degeneration, proliferative diabetic retinopathy, hypoxia-induced angiogenesis, rheumatoid arthritis, psoriasis, wound healing and others.

VEGF, an endothelial cell-specific mitogen, is a 34-45 kDa glycoprotein with a wide range of activities that include promotion of angiogenesis, enhancement of vascular-permeability and others. VEGF belongs to the platelet-derived growth factor (PDGF) family of growth factors with approximately 18% homology with the A and B chain of PDGF at the amino acid level. Additionally, VEGF contains the eight conserved cysteine residues common to all growth factors belonging to the PDGF family (Neufeld et al., supra). VEGF protein is believed to exist predominantly as disulfide-linked homodimers; monomers of VEGF have been shown to be inactive (Plouet et al., 1989 EMBO J. 8, 3801).

VEGF exerts its influence on vascular endothelial cells by binding to specific high-affinity cell surface receptors. Covalent cross-linking experiments with ¹²⁵I-labeled VEGF protein have led to the identification of three high molecular weight complexes of 225, 195 and 175 kDa presumed to be VEGF and VEGF receptor complexes (Vaisman *et al.*, 1990 *J. Biol. Chem.* 265, 19461). Based on these studies VEGF-specific receptors of 180, 150 and 130 kDa molecular mass were predicted. In endothelial cells, receptors of 150 and 130 kDa have been identified. The VEGF receptors belong to the superfamily of receptor tyrosine kinases (RTKs) characterized by a conserved cytoplasmic catalytic kinase domain and a hydrophilic kinase sequence. The extracellular domains of the VEGF receptors consist of seven immunoglobulin-like domains that are thought to be involved in VEGF binding functions.

The two most abundant and high-affinity receptors of VEGF are fit-1 (VEGFR1) (fms-like tyrosine kinase) cloned by Shibuya et al., 1990 Oncogene 5, 519 and KDR (VEGFR2) (kinase-insert-domain-containing receptor) cloned by Terman et al., 1991 Oncogene 6, 1677. The murine homolog of KDR, cloned by Mathews et al., 1991, Proc. Natl. Acad. Sci., USA, 88, 9026, shares 85% amino acid homology with KDR and is termed as fik-1 (fetal liver kinase-1). The high-affinity binding of VEGF to its receptors is modulated by cell surface-associated heparin and heparin-like molecules (Gitay-Goren et al., 1992 J. Biol. Chem. 267, 6093).

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VEGF expression has been associated with several pathological states such as tumor angiogenesis, several forms of blindness, rheumatoid arthritis, psoriasis and others. In addition, a number of studies have demonstrated that VEGF is both necessary and sufficient for neovascularization. Takashita et al., 1995 J. Clin. Invest. 93, 662, demonstrated that a single injection of VEGF augmented collateral vessel development in a rabbit model of ischemia. VEGF also can induce neovascularization when injected into the cornea. Expression of the VEGF gene in CHO cells is sufficient to confer tumorigenic potential to the cells. Kim et al., supra and Millauer et al., supra used monoclonal antibodies against VEGF or a dominant negative form of VEGFR2 receptor to inhibit tumor-induced neovascularization.

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During development, VEGF and its receptors are associated with regions of new vascular growth (Millauer et al., 1993 Cell 72, 835; Shalaby et al., 1993 J. Clin. Invest. 91, 2235). Furthermore, transgenic mice lacking either of the VEGF receptors are defective in blood vessel formation and these mice do not survive; VEGFR2 appears to be required for differentiation of endothelial cells, while VEGFR1 appears to be required at later stages of vessel formation (Shalaby et al., 1995 Nature 376, 62; Fung et al., 1995 Nature 376, 66). Thus, these receptors apparently need to be present to properly signal endothelial cells or their precursors to respond to vascularization-promoting stimuli.

Increasing evidence suggests that the VEGF family may also be involved with both the etiology and maintenance of peritoneal endometriosis. Peritoneal endometriosis is a significant debilitating gynecological problem of widespread prevalence. It is now generally accepted that the pathogenesis of peritoneal endometriosis involves the implantation of exfoliated endometrium. Maintenance of exfoliated endometrial tissue is dependent upon the generation and maintenance of an extensive blood supply both within and surrounding the ectopic tissue.

Endometriosis is a disease affecting an estimated 77 million women and teenagers worldwide. Endometriosis is a leading cause of infertility, chronic pelvic pain and hysterectomy. Endometriosis can be characterized when endometrial tissue (the tissue inside the uterus which builds up and is shed each month during menses) is found outside the uterus, in other areas of the body. The endometrial tissue can respond to hormonal commands each month and break down and bleed. However, unlike the endometrium, these tissue deposits have no way of leaving the body. The result is internal bleeding, degeneration of blood and tissue shed from the growths, inflammation of the surrounding areas, expression of irritating enzymes and formation of scar tissue. In addition, depending on the location of the growths,

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interference with the bowel, bladder, intestines and other areas of the pelvic cavity can occur. Endometrial tissue has even been found lodged in the skin and at other extrapelvic locations like the arm, leg and even brain.

Currently, the presence of Endometriosis can only be confirmed through surgery such as laparoscopy, but can be suspected based on symptoms, physical findings and diagnostic tests. Endometriosis can be treated in many different ways, both surgically and medically. Most commonly, surgery will be performed during which the disease will be excised, ablated, fulgarated, cauterized or otherwise removed, and adhesions will also be freed. Surgeries include but are not limited to laparoscopy; laparotomy; presacral and uterosacral and various levels of hysterectomies, where some or all of the reproductive organs are removed. Often, this method will only relieve the symptoms associated with growths on the reproductive organs, not the bowels or kidneys and related areas where Endometriosis can be present.

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There are several drugs used to treat Endometriosis that are utilized either alone or in combination with surgery. These include contraceptives, GnRH agonists, and/or synthetic hormones. GnRH agonists are commonly used on women in all stages of the disease and may sometimes have serious side affects. GnRH (gonadotropin releasing hormone) analogues are classified into 2 groups: agonists and antagonists. Agonists are commonly used in the treatment of Endometriosis by suppressing the manufacture of follicle stimulating hormone (FSH) and luteinizing hormone (LH), common hormones required in ovulation. When they are not secreted, the body will go into "pseudo-menopause," stalling the growth of more implants. However, these are again only stop-gap measures that can be utilized only for short term intervals. Once the body returns to it's normal state, the Endometriosis will again begin to implant itself.

Angiogenesis is likely to be involved in the pathogenesis of endometriosis. According to the transplantation theory, when the exfoliated endometrium is attached to the peritoneal layer, the establishment of a new blood supply is essential for the survival of the endometrial implant and development of endometriosis (Donnez et al., 1998, Hum. Reprod., 13, 1686-1690). Endometrial growth and repair after menstruation are associated with profound angiogenesis. Abnormalities in these processes result in excessive or unpredictable bleeding patterns and are common in many women. It is therefore important to understand which factors regulate normal endometrial angiogenesis. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that plays an important role in normal and pathological angiogenesis (Fasciani et al., 2000, Mol. Hum. Reprod., 6, 50-54; Sharkey et al., 2000, J. Clin. Endocrinol. Metab., 85, 402-409). Sources of this factor include the eutopic

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endometrium, ectopic endometriotic tissue and peritoneal fluid macrophages. Important to its etiology is the correct peritoneal environment in which the exfoliated endometrium is seeded and implants. Established ectopic tissue is then dependent on the peritoneal environment for its survival, an environment that supports angiogenesis. The increasing knowledge of the involvement of the VEGF family in endometriotic angiogenesis raises the possibility of novel approaches to its medical management, with particular focus on the anti-angiogenic control of the action of VEGF (McLaren, 2001, Hum. Reprod. Update, 6, 45-55).

Pavco et al., International PCT Publication No. WO 97/15662, describes methods and reagents for treating diseases or conditions related to levels of vascular endothelial growth factor receptor.

Robinson, International PCT Publication No. WO 95/04142, describes the use of certain antisense oligonucleotides targeted against VEGF RNA to inhibit VEGF expression.

Jellinek et al., 1994 Biochemistry 33, 10450 describe the use of specific VEGF-specific high-affinity RNA aptamers to inhibit the binding of VEGF to its receptors.

Rockwell and Goldstein, International PCT Publication No. WO 95/21868, describe the use of certain anti-VEGF receptor monoclonal antibodies to neutralize the effect of VEGF on endothelial cells.

Pappa, International PCT Publication No. WO 01/32920, describes inhibitors, including certain ribozyme and antisense nucleic acid molecules, of specific genes, including cathepsin D, AEBP-1, stromelysin-3, cystatin B, protease inhibitor 1, sFRP4, gelsolin, IGFBP-3, dual specificity phosphatase 1, PAEP, Ig gamma chain, ferritin, complement component 3, proalpha-1 type III collagen, proline 4-hydroxylase, alpha-2 type I collagen, claudin-4, melanoma adhesion protein, procollagen C-endopeptidase enhancer, nascent-polypeptide-associated complex alpha polypeptide, elongation factor 1 alpha (EF-1-alpha), vitamin D3 25 hydroxylase, CSRP-1, steroidogenic acute regulatory protein, apolipoprotein E, transcobalamin II, prosaposin, early growth response 1 (EGR1), ribosomal protein S6, adenosine dearninase RNA-specific protein, RAD21, guanine nucleotide binding protein beta polypeptide 2-like 1 (RACK1) and podocalyxin genes which are all differentially expressed in tissues within individual patients with endometriosis.

Labarbera et al., International PCT Publication No. WO 00/73416, describes specific antisense nucleic acid molecules targeting follicle-stimulating hormone receptor.

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Storella et al., International PCT Publication No. WO 99/63116, describes modulators of Prothymosin gene products for treating endometriosis, including certain ribozymes and antisense nucleic acid molecules.

Summary Of The Invention

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This invention features nucleic acid-based molecules, for example, enzymatic nucleic acid molecules, allozymes, antisense nucleic acids, 2-5A antisense chimeras, triplex forming oligonucleotides, decoy RNA, dsRNA, siRNA, aptamers, and antisense nucleic acids containing nucleic acid cleaving chemical groups, and methods to modulate vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptor (VEGFr) gene expression. Non-limiting examples of genes that encode vascular endothelial growth factor receptors of the invention include VEGFR1, VEGFR2 or combinations thereof. In particular, the instant invention features nucleic acid-based molecules and methods that modulate the expression of vascular endothelial growth factor and/or vascular endothelial growth factor receptors, such as VEGFR1 and/or VEGFR2, that are useful in preventing, treating, controlling, and/or diagnosing angiogenesis related diseases and conditions, including but not limited to tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and female reproductive disorders and conditions, including but not limited to endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), and menopausal dysfunction.

In one embodiment, the invention features one or more nucleic acid-based molecules and methods that independently or in combination modulate the expression of gene(s) encoding vascular endothelial growth factor receptors. Specifically, the present invention features nucleic acid molecules that modulate the expression of VEGF (for example Genbank Accession No. NM_003376), VEGFR1 receptor (for example Genbank Accession No. NM_002019), and VEGFR2 receptor (for example Genbank Accession No. NM_002253) that are useful in preventing, treating, controlling, and/or diagnosing tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and female reproductive disorders and conditions, including but not limited to

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endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), and menopausal dysfunction.

In one embodiment, the present invention features a compound having Formula I: (SEQ ID NO: 5977)

5' gsasgsusugcUGAuGagg ccgaaa ggccGaaAgucugB 3'

wherein each a is 2'-O-methyl adenosine nucleotide, each g is a 2'-O-methyl guanosine nucleotide, each c is a 2'-O-methyl cytidine nucleotide, each u is a 2'-O-methyl uridine nucleotide, each A is adenosine, each G is guanosine, each s individually represents a phosphorothioate internucleotide linkage, U is 2'-deoxy-2'-C-allyl uridine, and B is an inverted deoxyabasic moiety. This compound is also referred to as ANGIOZYMETM ribozyme.

In another embodiment, the present invention features a compound having Formula II: (SEQ ID NO: 5978).

5'-usascs asau ucU GAu Gag gcg aaa gcc Gaa Aag aca aB-3'

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wherein each a is 2'-O-methyl adenosine nucleotide, each g is a 2'-O-methyl guanosine nucleotide, each c is a 2'-O-methyl cytidine nucleotide, each u is a 2'-O-methyl uridine nucleotide, each A is adenosine, each G is guanosine, each s individually represents a phosphorothicate internucleotide linkage, \underline{U} is 2'-deoxy-2'-C-allyl uridine, and B is an inverted deoxyabasic moiety.

In one embodiment, the invention features a composition comprising a nucleic acid molecule of the invention in a pharmaceutically acceptable carrier. In another embodiment, the invention features a composition comprising a compound of Formula I and/or Formula II in a pharmaceutically acceptable carrier or diluent.

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In one embodiment, the invention features a method of administering to a cell, for example a mammalian cell, including a human cell, a nucleic acid molecule of the invention comprising contacting the cell with the nucleic acid molecule under conditions suitable for administration, for example in the presence of a delivery reagent such as a lipid, cationic lipid, phospholipid, or liposome. In another embodiment, the invention features a method of administering to a cell, for example a mammalian cell, including a human cell, a compound of Formula I and/or Formula II comprising contacting the cell with the compound under

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conditions suitable for administration, for example in the presence of a delivery reagent such as a lipid, cationic lipid, phospholipid, or liposome.

In one embodiment, the present invention features a mammalian cell comprising a nucleic acid molecule of the invention, wherein the mammalian cell is, for example, a human cell. In another embodiment, the present invention also features a mammalian cell comprising the compound of Formula I and/or Formula II, wherein the mammalian cell is, for example, a human cell.

In one embodiment, the invention features a method of inhibiting angiogenesis, for example tumor angiogenesis, or ocular indications such as diabetic retinopathy, or age related macular degeneration, or endometrial neovascularization, in a subject comprising contacting the subject with a nucleic acid molecule of the invention, under conditions suitable for the inhibition. In another embodiment, the invention features a method of inhibiting angiogenesis, for example tumor angiogenesis, or ocular indications such as diabetic retinopathy, or age related macular degeneration, or endometrial neovascularization, in a subject, comprising contacting the subject with a compound of Formula I and/or Formula II, under conditions suitable for the inhibition.

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In another embodiment, the invention features a method of treatment of a subjecthaving an ocular condition associated with the increased level of a VEGF receptor, for example diabetic retinopathy, or age related macular degeneration, comprising contacting cells of the subjectwith a nucleic acid molecule, such as an enzymatic nucleic acid molecule targeted against a VEGF receptor RNA, e.g., molecule according to Formula I and/or II, under conditions suitable for the treatment.

In another embodiment, the invention features a method of treatment of a subjecthaving a condition associated with an increased level of VEGR and/or a VEGF receptor, for example tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, ocular diseases or ocular indications such as diabetic retinopathy, or age related macular degeneration, rhuematoid arthritis, psoriasis endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), or menopausal dysfunction, comprising contacting cells of the subject with a nucleic acid molecule of the invention, such as a compound of Formula I and/or Formula II, under conditions suitable for the treatment.

In yet another embodiment, the inventive method of treatment further comprises the use of one or more drug therapies under conditions suitable for the treatment. Non-limiting

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examples of other drug therapies that can be used in combination with nucleic acid molecules of the invention include to 5-fluoro uridine, Leucovorin, Irinotecan (CAMPTOSAR® or CPT-11 or Camptothecin-11 or Campto), Paclitaxel, or Carboplatin, GnRH (gonadotropin releasing hormone) agonists, Lupron Depot (Leuprolide Acetate), Synarel (naferalin acetate), Zolodex (goserelin acetate), Suprefact (buserelin acetate), Danazol, or oral contraceptives including but not limited to Depo-Provera or Provera (medroxyprogesterone acetate), or any other estrogen/progesterone contraceptive.

In one embodiment, the invention features a method of administering to a mammal, for example a human, a nucleic acid molecule of the invention comprising contacting the mammal with the nucleic acid molecule under conditions suitable for the administration, for example, in the presence of a delivery reagent such as a lipid, cationic lipid, phospholipid, or liposome. In another embodiment, the invention features a method of administering to a mammal, for example a human, a compound of Formula I and/or Formula II comprising contacting the mammal with the compound under conditions suitable for the administration, for example, in the presence of a delivery reagent such as a lipid, cationic lipid, phospholipid, or liposome.

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In one embodiment, the invention features a nucleic acid molecule which down regulates expression of a vascular endothelial growth factor (VEGF) and/or vascular endothelial growth factor receptor (VEGFr) gene, for example, wherein the VEGFr gene comprises VEGFR1 or VEGFR2 and any combination thereof.

In one embodiment, a nucleic acid molecule of the invention, such as an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups, is adapted to treat, control and/or diagnose tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, ocular diseases or ocular indications, such as diabetic retinopathy, or age related macular degeneration, rhuematoid arthritis, psoriasis endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), or menopausal dysfunction.

Such nucleic acid molecules are also useful for the prevention of the diseases and conditions including diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, verruca vulgaris, angiofibroma of tuberous sclerosis, port-wine stains, Sturge Weber syndrome, Kippel-Trenaumay-Weber syndrome, Osler-Weber-Rendu syndrome

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and other diseases or conditions that are related to the levels of VEGFR1 or VEGFR2 in a cell or tissue.

In another embodiment, the invention features a composition in a pharmaceutically acceptable carrier or diluent, comprising the nucleic acid molecule of the instant invention.

In another embodiment, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention is adapted for birth control.

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In one embodiment, an enzymatic nucleic acid molecule of the invention is in a hammerhead, Inozyme, Zinzyme, DNAzyme, Amberzyme, or G-cleaver configuration.

In one embodiment, an enzymatic nucleic acid molecule of the invention comprises between 8 and 100 bases complementary to RNA of VEGFR1 and/or VEGFR2 gene. In another embodiment, an enzymatic nucleic acid molecule of the invention comprises between 14 and 24 bases complementary to RNA of VEGFR1 and/or VEGFR2 gene.

In one embodiment, a siRNA molecule of the invention comprises a double stranded RNA wherein one strand of the RNA is complementary to RNA of a VEGFR1 and/or VEGFR2 gene. In another embodiment, a siRNA molecule of the invention comprises a double stranded RNA wherein one strand of the RNA comprises a portion of a sequence of RNA having a VEGFR1 and/or VEGFR2 sequence. In yet another embodiment, a siRNA molecule of the invention comprises a double stranded RNA wherein both strands of RNA are connected by a non-nucleotide linker. Alternately, a siRNA molecule of the invention comprises a double stranded RNA wherein both strands of RNA are connected by a nucleotide linker, such as a loop or stem loop structure.

In one embodiment, a single strand component of a siRNA molecule of the invention is from about 14 to about 50 nucleotides in length. In another embodiment, a single strand component of a siRNA molecule of the invention is about 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides in length. In yet another embodiment, a single strand component of a siRNA molecule of the invention is about 23 nucleotides in length. In one embodiment, a siRNA molecule of the invention is from about 28 to about 56 nucleotides in length. In another embodiment, a siRNA molecule of the invention is about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 nucleotides in length. In yet another embodiment, a siRNA molecule of the invention is about 46 nucleotides in length.

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In one embodiment, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention is chemically synthesized.

In another embodiment, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention comprises at least one 2'-sugar modification.

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In another embodiment, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acids containing nucleic acid cleaving chemical groups of the invention comprises at least one nucleic acid base modification.

In another embodiment, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention comprises at least one phosphate backbone modification.

In one embodiment, the invention features a mammalian cell, for example a human cell, comprising a nucleic acid molecule of the invention.

In another embodiment, the invention features a method of reducing VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 expression or activity in a cell comprising contacting the cell with a nucleic acid molecule of the invention that modulates the expression and/or activity of VEGF and/or VEGFr, under conditions suitable for the reduction.

In another embodiment, a method of treatment of a subject having a condition associated with the level of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 is featured, wherein the method further comprises the use of one or more drug therapies under conditions suitable for the treatment.

In one embodiment, the invention features a method for treatment of a subject having tumor angiogenesis, tumor angiogenesis, cancers including but not limited to tumor and cancer types shown under Diagnosis in Table III, ocular diseases or ocular indications such as diabetic retinopathy, or age related macular degeneration, rhuematoid arthritis, psoriasis and/or endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular

menstrual cycles, ovulation, premenstrual syndrome (PMS), or menopausal dysfunction, comprising administering to the subject a nucleic acid molecule of the invention that modulates the expression and/or activity of VEGF and/or VEGFr under conditions suitable for the treatment.

In another embodiment, the invention features a method for birth control in a subject comprising administering to the subject a nucleic acid molecule of the invention that modulates the expression and/or activity of VEGF and/or VEGFr under conditions suitable for the treatment.

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In another embodiment, the invention features a method of cleaving RNA encoded by a VEGF, VEGFR1 and/or VEGFR2 gene comprising contacting an enzymatic nucleic acid molecule of the invention having endonuclease activity with RNA encoded by a VEGFR1 and/or VEGFR2 gene under conditions suitable for the cleavage, for example, wherein the cleavage is carried out in the presence of a divalent cation, such as Mg²⁺.

In one embodiment, a nucleic acid molecule of the invention comprises a cap structure, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative, wherein the cap structure is at the 5'-end, or 3'-end, or both the 5'-end and the 3'-end of the enzymatic nucleic acid molecule.

In another embodiment, a nucleic acid molecule of the invention comprises a cap structure, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative, wherein the cap structure is at the 5'-end, or 3'-end, or both the 5'-end and the 3'-end of the antisense nucleic acid molecule.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one nucleic acid molecule of the invention such that the vector allows expression of the nucleic acid molecule.

In another embodiment, the invention features a mammalian cell, for example, a human cellcomprising an expression vector of the invention.

In yet another embodiment, an expression vector of the invention further comprises a sequence for a nucleic acid molecule complementary to RNA encoded by a VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 gene.

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In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more nucleic acid molecules of the invention, which can be the same or different.

In another embodiment, the invention features a method for treatment or control of tumor angiogenesis, cancers such as breast cancer, hung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and/or endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), or menopausal dysfunction, comprising administering to a subject a nucleic acid molecule of the invention that modulates the expression and/or activity of VEGF and/or VEGFr, such as an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention, under conditions suitable for the treatment, including administering to the subject one or more other therapies, for example, 5-fluoro uridine, Leucovorin, Irinotecan (CAMPTOSAR® or CPT-11 or Camptothecin-11 or Campto), Paclitaxel, or Carboplatin.GnRH (gonadotropin releasing hormone) agonists, Lupron Depot (Leuprolide Acetate), Synarel (naferalin acetate), Zolodex (goserelin acetate), Suprefact (buserelin acetate), Danazol, or oral contraceptives including but not limited to Depo-Provera or Provera (medroxyprogesterone acetate), or any other estrogen/progesterone contraceptive.

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In one embodiment, the method of treatment features a nucleic acid molecule of the invention, such as an enzymatic nucleic acid or antisense nucleic acid molecule, that comprises at least five ribose residues, at least ten 2'-O-methyl modifications, and a 3'- end modification, such as a 3'-3' inverted abasic moiety. In another embodiment, a nucleic acid molecule of the invention further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

In another embodiment, the invention features a method of administering to a mammal, for example a human, an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention, comprising contacting the mammal with the nucleic acid molecule under conditions suitable for the administration, for example, in the presence of a delivery reagent such as a lipid, cationic lipid, phospholipid, or liposome.

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In yet another embodiment, the invention features a method of administering to a mammal an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acid containing nucleic acid cleaving chemical groups of the invention in conjunction with other therapies, comprising contacting the mammal, for example a human, with the nucleic acid molecule and the other therapy under conditions suitable for the administration.

In another embodiment, other therapies contemplated by the instant invention that can be used in conjunction with the nucleic acid molecules of the instant invention include, but are not limited to, 5-fluoro uridine, Leucovorin, Irinotecan (CAMPTOSAR® or CPT-11 or Camptothecin-11 or Campto), Paclitaxel, or Carboplatin, GnRH (gonadotropin releasing hormone) agonists, Lupron Depot (Leuprolide Acetate), Synarel (naferalin acetate), Zolodex (goserelin acetate), Suprefact (buserelin acetate), Danazol, or oral contraceptives including but not limited to Depo-Provera or Provera (medroxyprogesterone acetate), or other estrogen/progesterone contraceptive.

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In one embodiment, the invention features the use of an enzymatic nucleic acid molecule, to down-regulate the expression of VEGFR1 and/or VEGFR2 genes in the treatment or control of tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and/or endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), or menopausal dysfunction. Such enzymatic nucleic acid molecule can be in the hammerhead, NCH, G-cleaver, Amberzyme, Zinzyme, and/or DNAzyme motif.

In another embodiment, the invention features the use of an enzymatic nucleic acid moleculeto down-regulate the expression of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 genes, as a method of birth control. Such enzymatic nucleic acid molecule can be in the hammerhead, NCH, G-cleaver, Amberzyme, Zinzyme, and/or DNAzyme motif. In one embodiment, the nucleic acid molecules of the invention have complementarity to the substrate sequences in Tables V and VI. Examples of enzymatic nucleic acid molecules of the invention are shown in Tables V and VI. Examples of such enzymatic nucleic acid molecules consist essentially of sequences defined in these Tables.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of nucleic acids or equivalent nucleic acids encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, such as VEGFR1, VEGFR2

and/or fik-1, is reduced below that observed in the absence of the nucleic acid molecules of the invention. In one embodiment, inhibition, down-regulation or reduction with enzymatic nucleic acid molecule preferably is below that level observed in the presence of an enzymatically inactive or attenuated molecule that is able to bind to the same site on the target nucleic acid, but is unable to cleave that nucleic acid. In another embodiment, inhibition, down-regulation, or reduction with antisense oligonucleotides is preferably below that level observed in the presence of, for example, an oligonucleotide with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 with the nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

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By "up-regulate" is meant that the expression of a gene, or level of nucleic acids or equivalent nucleic acids encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits, such as VEGFR1 and/or VEGFR2, is greater than that observed in the absence of the nucleic acid molecules of the invention. For example, the expression of a gene, such as VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 gene, can be increased in order to treat, prevent, ameliorate, or modulate a pathological condition caused or exacerbated by an absence or low level of gene expression.

By "modulate" is meant that the expression of a gene, or level of nucleic acids or equivalent nucleic acids encoding one or more proteins or protein subunits, or activity of one or more proteins protein subunit(s) is up-regulated or down-regulated, such that the expression, level, or activity is greater than or less than that observed in the absence of the nucleic acid molecules of the invention.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave a target nucleic acid. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave a nucleic acid and thereby inactivate a target nucleic acid molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target nucleic acid and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% can also be useful in this invention (see for example Werner and Uhlenbeck, 1995, Nucleic Acids Research, 23, 2092-2096; Hammann et al., 1999, Antisense and Nucleic Acid Drug Dev., 9, 25-31). The nucleic acids can be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such

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as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving and/or ligation activity to the molecule (Cech et al., U.S. Patent No. 4,987,071; Cech et al., 1988, 260 JAMA 3030).

Several varieties of naturally-occurring enzymatic nucleic acids are known presently. Each can catalyze the hydrolysis of nucleic acid phosphodiester bonds in trans (and thus can cleave other nucleic acid molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target nucleic acid. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target nucleic acid. Thus, the enzymatic nucleic acid first recognizes and then binds a target nucleic acid through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target nucleic acid. Strategic cleavage of such a target nucleic acid will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its nucleic acid target, it is released from that nucleic acid to search for another target and can repeatedly bind and cleave new targets. Thus, a single ribozyme molecule is able to cleave many molecules of target nucleic acid. In addition, the ribozyme is a highly specific inhibitor of gene expression, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target nucleic acid, but also on the mechanism of target nucleic acid cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme.

In one embodiment of the inventions described herein, an enzymatic nucleic acid molecule of the invention is formed in a hammerhead or hairpin motif, but can also be formed in the motif of a hepatitis delta virus, group I intron, group II intron or RNase P RNA (in association with an RNA guide sequence), Neurospora VS RNA, DNAzymes, NCH cleaving motifs, or G-cleavers. Examples of such hammerhead motifs are described by Dreyfus, supra, Rossi et al., 1992, AIDS Research and Human Retroviruses 8, 183; of hairpin motifs

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by Hampel et al., EP0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, Feldstein et al., 1989, Gene 82, 53, Haseloff and Gerlach, 1989, Gene, 82, 43, and Hampel et al., 1990 Nucleic Acids Res. 18, 299; Chowrira & McSwiggen, US. Patent No. 5,631,359; an examples of a hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; examples of RNase P motifs are described by Guerrier-Takada et al., 1983 Cell 35, 849; Forster and Altman, 1990, Science 249, 783; Li and Altman, 1996, Nucleic Acids Res. 24, 835; examples of Neurospora VS RNA ribozyme motifs are described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799; Guo and Collins, 1995, EMBO. J. 14, 363); examples of Group II introns are described by Griffin et al., 1995, Chem. Biol. 2, 761; Michels and Pyle, 1995, Biochemistry 34, 2965; Pyle et al., International PCT Publication No. WO 96/22689; an example of a Group I intron is described by Cech et al., U.S. Patent 4,987,071; and examples of DNAzymes are described by Usman et al., International PCT Publication No. WO 95/11304; Chartrand et al., 1995, NAR 23, 4092; Breaker et al., 1995, Chem. Bio. 2, 655; Santoro et al., 1997, PNAS 94, 4262, and Beigelman et al., International PCT publication No. WO 99/55857. NCH cleaving motifs are described in Ludwig & Sproat, International PCT Publication No. WO 98/58058; and G-cleavers are described in Kore et al., 1998, Nucleic Acids Research 26, 4116-4120 and Eckstein et al., International PCT Publication No. WO 99/16871. Additional motifs such as the Aptazyme (Breaker et al., WO 98/43993), Amberzyme (Beigelman et al., U.S. Serial No. 09/301,511) and Zinzyme (Figure 7) (Beigelman et al., U.S. Serial No. 09/918,728), all included by reference herein including drawings, can also be used in the present invention. These specific motifs or configurations are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it have a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a RNA cleaving activity to the molecule (Cech et al., U.S. Patent No. 4,987,071).

By "nucleic acid molecule" as used herein is meant a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and can comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

By "enzymatic portion" or "catalytic domain" is meant that portion/region of a enzymatic nucleic acid molecule essential for cleavage of a nucleic acid substrate (for example see Figure 6).

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By "substrate binding arm" or "substrate binding domain" is meant that portion/region of a enzymatic nucleic acid which is able to interact, for example via complementarity (i.e., able to base-pair with), with a portion of its substrate. Preferably, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 can be base-paired (see for example Werner and Uhlenbeck, 1995, Nucleic Acids Research, 23, 2092-2096; Hammann et al., 1999, Antisense and Nucleic Acid Drug Dev., 9, 25-31). Examples of such arms are shown generally in Figures 6-8. That is, these arms contain sequences within a enzymatic nucleic acid which are intended to bring enzymatic nucleic acid and target nucleic acid together through complementary base-pairing interactions. An enzymatic nucleic acid of the invention can have binding arms that are contiguous or non-contiguous and can be of varying lengths. The length of the binding arm(s) are preferably greater than or equal to four nucleotides and of sufficient length to stably interact with the target nucleic acid; preferably 12-100 nucleotides; more preferably 14-24 nucleotides long (see for example Werner and Uhlenbeck, supra; Hamman et al., supra; Hampel et al., EP0360257; Berzal-Herranz et al., 1993, EMBO J., 12, 2567-73) or between 8 and 14 nucleotides long. If two binding arms are chosen, the design is such that the length of the binding arms are symmetrical (i.e., each of the binding arms is of the same length; e.g., four and four, five and five nucleotides, or six and six nucleotides, or seven and seven nucleotides long) or asymmetrical (i.e., the binding arms are of different length; e.g., three and five, six and three nucleotides; three and six nucleotides long; four and five nucleotides long; four and six nucleotides long; four and seven nucleotides long; and the like).

By "Inozyme" or "NCH" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as NCH Rz in Figure 6 and in Ludwig et al., International PCT Publication No. WO 98/58058 and US Patent Application Serial No. 08/878,640. Inozymes possess endonuclease activity to cleave nucleic acid substrates having a cleavage triplet NCH/, where N is a nucleotide, C is cytidine and H is adenosine, uridine or cytidine, and "/" represents the cleavage site. H is used interchangeably with X. Inozymes can also possess endonuclease activity to cleave nucleic acid substrates having a cleavage triplet NCN/, where N is a nucleotide, C is cytidine, and "/" represents the cleavage site. "T" in Figure 6 represents an Inosine nucleotide, preferably a ribo-Inosine or xylo-Inosine nucleoside.

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By "G-cleaver" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as G-cleaver Rz in Figure 6 and in Eckstein et al., US 6,127,173. G-cleavers possess endonuclease activity to cleave nucleic acid substrates having a cleavage triplet NYN/, where N is a nucleotide, Y is uridine or cytidine and "/"

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represents the cleavage site. G-cleavers can be chemically modified as is generally shown in Figure 6.

By "amberzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Beigelman et al., International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/476,387. Amberzymes possess endonuclease activity to cleave nucleic acid substrates having a cleavage triplet NG/N, where N is a nucleotide, G is guanosine, and "/" represents the cleavage site. Amberzymes can be chemically modified to increase nuclease stability through substitutions using modified nucleotides. In addition, differing nucleoside and/or non-nucleoside linkers can be used to substitute the 5'-gaaa-3' loops shown in the figure. Amberzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

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By "zinzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Figure 7 and in Beigelman et al., International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/918,728. Zinzymes possess endonuclease activity to cleave nucleic acid substrates having a cleavage triplet including but not limited to YG/Y, where Y is uridine or cytidine, and G is guanosine and "/" represents the cleavage site. Zinzymes can be chemically modified to increase nuclease stability through substitutions as are generally shown in Figure 7, including substituting 2'-O-methyl guanosine nucleotides for guanosine nucleotides. In addition, differing nucleotide and/or non-nucleotide linkers can be used to substitute the 5'-gaaa-2' loop shown in the figure. Zinzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By 'DNAzyme' is meant, an enzymatic nucleic acid molecule that does not require the presence of a 2'-OH group within its own nucleic acid sequence for activity. In particular embodiments the enzymatic nucleic acid molecule can have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. DNAzymes can be synthesized chemically or expressed endogenously in vivo, by means of a single stranded DNA vector or equivalent thereof. An example of a DNAzyme is shown in Figure 8 and is generally reviewed in Usman et al., US patent No., 6,159,714; Chartrand et al., 1995, NAR 23, 4092; Breaker et al., 1995, Chem. Bio. 2, 655; Santoro et al., 1997, PNAS 94, 4262; Breaker, 1999, Nature Biotechnology, 17, 422-423; and

Santoro et. al., 2000, J. Am. Chem. Soc., 122, 2433-39. The "10-23" DNAzyme motif is one particular type of DNAzyme that was evolved using in vitro selection, see Santoro et al., supra and as generally described in Joyce et al., US 5,807,718. Additional DNAzyme motifs can be selected for using techniques similar to those described in these references, and hence, are within the scope of the present invention.

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By "sufficient length" is meant a nucleic acid molecule of the invention is long enough to provide the intended function under the expected condition. For example, a nucleic acid molecule of the invention needs to be of "sufficient length" to provide stable interaction with a target nucleic acid molecule under the expected binding conditions and environment. In another non-limiting example, for the binding arms of an enzymatic nucleic acid, "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected reaction conditions and environment. The binding arms are not so long as to prevent useful turnover of the nucleic acid molecule.

By "stably interact" is meant interaction of an oligonucleotides with target nucleic acid (e.g., by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions) that is sufficient to the intended purpose (e.g., cleavage of target nucleic acid by an enzyme).

By "equivalent" RNA to VEGF, VEGFR1 and/or VEGFR2 is meant to include nucleic acid molecules having homology (partial or complete) to a nucleic acid encoding VEGF, VEGFR1 and/or VEGFR2 proteins or encoding proteins with similar function as VEGF, VEGFR1 and/or VEGFR2 proteins in various organisms, including human, rodent, primate, rabbit, pig, protozoans, fungi, plants, and other microorganisms and parasites. The equivalent nucleic acid sequence also includes, in addition to the coding region, regions such as 5'-untranslated region, 3'-untranslated region, intron-exon junction and the like.

By "homology" is meant the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

By "antisense nucleic acid", it is meant a non-enzymatic nucleic acid molecule that binds to target nucleic acid by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 Nature 365, 566) interactions and alters the activity of the target nucleic acid (for a review, see Stein and Cheng, 1993 Science 261, 1004 and Woolf et al., US patent No. 5,849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule

forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, an antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk et al., 1999, J. Biol. Chem., 274, 21783-21789, Delihas et al., 1997, Nature, 15, 751-753, Stein et al., 1997, Antisense N. A. Drug Dev., 7, 151, Crooke, 2000, Methods Enzymol., 313, 3-45; Crooke, 1998, Biotech. Genet. Eng. Rev., 15, 121-157, Crooke, 1997, Ad. Pharmacol., 40, 1-49. In addition, antisense DNA can be used to target nucleic acid by means of DNA-RNA interactions, thereby activating RNase H, which digests the target nucleic acid in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target nucleic acid. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof.

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By "RNase H activating region" is meant a region (generally greater than or equal to 4-25 nucleotides in length, preferably from 5-11 nucleotides in length) of a nucleic acid molecule capable of binding to a target nucleic acid to form a non-covalent complex that is recognized by cellular RNase H enzyme (see for example Arrow et al., US 5,849,902; Arrow et al., US 5,989,912). The RNase H enzyme binds to a nucleic acid molecule-target nucleic acid complex and cleaves the target nucleic acid sequence. The RNase H activating region comprises, for example, phosphodiester, phosphorothioate (preferably at least four of the nucleotides are phosphorothiote substitutions; more specifically, 4-11 of the nucleotides are phosphorothiote substitutions); phosphorodithioate, 5'-thiophosphate, or methylphosphonate backbone chemistry or a combination thereof. In addition to one or more backbone chemistries described above, the RNase H activating region can also comprise a variety of sugar chemistries. For example, the RNase H activating region can comprise deoxyribose, arabino, fluoroarabino or a combination thereof, nucleotide sugar chemistry. Those skilled in the art will recognize that the foregoing are non-limiting examples and that any combination of phosphate, sugar and base chemistry of a nucleic acid that supports the activity of RNase H enzyme is within the scope of the definition of the RNase H activating region and the instant invention.

By "2-5A antisense chimera" is meant an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target nucleic acid in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which, in turn, cleaves the target nucleic acid (Torrence et al., 1993 Proc. Natl. Acad. Sci. USA 90, 1300;

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Silverman et al., 2000, Methods Enzymol., 313, 522-533; Player and Torrence, 1998, Pharmacol. Ther., 78, 55-113).

By "triplex forming oligonucleotides" is meant an oligonucleotide that can bind to a double-stranded polynucleotide, such as DNA, in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 Proc. Natl. Acad. Sci. USA 89, 504; Fox, 2000, Curr. Med. Chem., 7, 17-37; Praseuth et. al., 2000, Biochim. Biophys. Acta, 1489, 181-206).

By "gene" it is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including but not limited to structural genes encoding a polypeptide.

The term "complementarity" as used herein refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., enzymatic nucleic acid cleavage, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, CSH Symp. Quant. Biol. LII pp.123-133; Frier et al., 1986, Proc. Nat. Acad. Sci. USA 83:9373-9377; Turner et al., 1987, J. Am. Chem. Soc. 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" or "2'-OH" is meant a nucleotide with a hydroxyl group at the 2' position of a β -D-ribo-furanose moiety.

By "nucleic acid decoy molecule", or "decoy" as used herein is meant a nucleic acid molecule that mimics the natural binding domain for a ligand. The decoy therefore competes with the natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a

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"decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990, Cell, 63, 601-608).

By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that is distinct from sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. Similarly, the nucleic acid molecules of the instant invention can bind to VEGFR1 or VEGFR2 receptors to block activity of the receptor. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art, see for example Gold et al., US 5,475,096 and 5,270,163; Gold et al., 1995, Annu. Rev. Biochem., 64, 763; Brody and Gold, 2000, J. Biotechnol., 74, 5; Sun, 2000, Curr. Opin. Mol. Ther., 2, 100; Kusser, 2000, J. Biotechnol., 74, 27; Hermann and Patel, 2000, Science, 287, 820; and Jayasena, 1999, Clinical Chemistry, 45, 1628.

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The term "double stranded RNA" or "dsRNA" as used herein refers to a double stranded RNA molecule capable of RNA interference "RNAi", including short interfering RNA "siRNA" see for example Bass, 2001, Nature, 411, 428-429; Elbashir et al., 2001, Nature, 411, 494-498; and Kreutzer et al., International PCT Publication No. WO 00/44895; Zernicka-Goetz et al., International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck et al., International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li et al., International PCT Publication No. WO 00/44914.

By "nucleic acid sensor molecule" or "allozyme" as used herein is meant a nucleic acid molecule comprising an enzymatic domain and a sensor domain, where the enzymatic nucleic acid domain's ability to catalyze a chemical reaction is dependent on the interaction with a target signaling molecule, such as a nucleic acid, polynucleotide, oligonucleotide, peptide, polypeptide, or protein, for example VEGF, VEGFR1 and/or VEGFR2. The introduction of chemical modifications, additional functional groups, and/or linkers, to the nucleic acid sensor molecule can provide enhanced catalytic activity of the nucleic acid sensor molecule, increased binding affinity of the sensor domain to a target nucleic acid, and/or improved nuclease/chemical stability of the nucleic acid sensor molecule, and are

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hence within the scope of the present invention (see for example Usman et al., US Patent Application No. 09/877,526, George et al., US Patent Nos. 5,834,186 and 5,741,679, Shih et al., US Patent No. 5,589,332, Nathan et al., US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker et al., International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger et al., US Patent Application Serial No. 09/205,520).

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By "sensor component" or "sensor domain" of the nucleic acid sensor molecule as used herein is meant, a nucleic acid sequence (e.g., RNA or DNA or analogs thereof) which interacts with a target signaling molecule, for example a nucleic acid sequence in one or more regions of a target nucleic acid molecule or more than one target nucleic acid molecule, and which interaction causes the enzymatic nucleic acid component of the nucleic acid sensor molecule to either catalyze a reaction or stop catalyzing a reaction. In the presence of target signaling molecule of the invention, such as VEGF, VEGFR1 and/or VEGFR2, the ability of the sensor component, for example, to modulate the catalytic activity of the nucleic acid sensor molecule, is inhibited or diminished. The sensor component can comprise recognition properties relating to chemical or physical signals capable of modulating the nucleic acid sensor molecule via chemical or physical changes to the structure of the nucleic acid sensor molecule. The sensor component can be derived from a naturally occurring nucleic acid binding sequence, for example, RNAs that bind to other nucleic acid sequences in vivo. Alternately, the sensor component can be derived from a nucleic acid molecule (aptamer) which is evolved to bind to a nucleic acid sequence within a target nucleic acid molecule (see for example Gold et al., US 5,475,096 and 5,270,163). The sensor component can be covalently linked to the nucleic acid sensor molecule, or can be non-covalently associated. A person skilled in the art will recognize that all that is required is that the sensor component is able to selectively inhibit the activity of the nucleic acid sensor molecule to catalyze a reaction.

By "target molecule" or "target signaling molecule" is meant a molecule capable of interacting with a nucleic acid sensor molecule, specifically a sensor domain of a nucleic acid sensor molecule, in a manner that causes the nucleic acid sensor molecule to be active or inactive. The interaction of the signaling agent with a nucleic acid sensor molecule can result in modification of the enzymatic nucleic acid component of the nucleic acid sensor molecule via chemical, physical, topological, or conformational changes to the structure of the molecule, such that the activity of the enzymatic nucleic acid component of the nucleic acid sensor molecule is modulated, for example is activated or deactivated. Signaling agents can comprise target signaling molecules such as macromolecules, ligands, small molecules,

metals and ions, nucleic acid molecules including but not limited to RNA and DNA or analogs thereof, proteins, peptides, antibodies, polysaccharides, lipids, sugars, microbial or cellular metabolites, pharmaceuticals, and organic and inorganic molecules in a purified or unpurified form, for example VEGF, VEGFR1 and/or VEGFR2.

The term "triplex forming oligonucleotides" as used herein refers to an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such a triple helix structure has been shown to inhibit transcription of a targeted gene (Duval-Valentin et al., 1992 Proc. Natl. Acad. Sci. USA 89, 504; Fox, 2000, Curr. Med. Chem., 7, 17-37; Praseuth et. al., 2000, Biochim. Biophys. Acta, 1489, 181-206).

The nucleic acid molecules that modulate the expression of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 specific nucleic acids, represent a novel therapeutic approach to treat or control a variety of angiogenesis related disorders and conditions, including but not limited to tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and/or endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), and/or menopausal dysfunction. The nucleic acid molecules that modulate the expression of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 specific nucleic acids also represent a novel approach to control ovulation or embryonic implantation and therefore provide a novel means of birth control.

In one embodiment of the present invention, a nucleic acid molecule of the instant invention can be between 12 and 100 nucleotides in length. An exemplary enzymatic nucleic acid molecule of the invention is shown as Formula I and/or Formula II. For example, enzymatic nucleic acid molecules of the invention are preferably between 15 and 50 nucleotides in length, more preferably between 25 and 40 nucleotides in length, e.g., 34, 36, or 38 nucleotides in length (for example see Jarvis et al., 1996, J. Biol. Chem., 271, 29107-29112). Exemplary DNAzymes of the invention are preferably between 15 and 40 nucleotides in length, more preferably between 25 and 35 nucleotides in length, e.g., 29, 30, 31, or 32 nucleotides in length (see for example Santoro et al., 1998, Biochemistry, 37, 13330-13342; Chartrand et al., 1995, Nucleic Acids Research, 23, 4092-4096). Exemplary antisense molecules of the invention are preferably between 15 and 75 nucleotides in length, more preferably between 20 and 35 nucleotides in length, e.g., 25, 26, 27, or 28 nucleotides in length (see for example Woolf et al., 1992, PNAS., 89, 7305-7309; Milner et al., 1997, Nature Biotechnology, 15, 537-541). Exemplary triplex forming oligonucleotide molecules of the invention are preferably between 10 and 40 nucleotides in length, more preferably

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between 12 and 25 nucleotides in length, e.g., 18, 19, 20, or 21 nucleotides in length (see for example Maher et al., 1990, Biochemistry, 29, 8820-8826; Strobel and Dervan, 1990, Science, 249, 73-75). Those skilled in the art will recognize that all that is required is that the nucleic acid molecule be of length and conformation sufficient and suitable for the nucleic acid molecule to catalyze a reaction contemplated herein. The length of the nucleic acid molecules of the instant invention are not limiting within the general limits stated.

In a preferred embodiment, a nucleic acid molecule that modulates, for example, down-regulates, VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 replication or expression comprises between 8 and 100 bases complementary to a nucleic acid molecule of VEGFR1 and/or VEGFR2. More preferably, a nucleic acid molecule that modulates VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 replication or expression comprises between 14 and 24 bases complementary to a nucleic acid molecule of VEGFR1 and/or VEGFR2.

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The invention provides a method for producing a class of nucleic acid—based gene modulating agents which exhibit a high degree of specificity for the nucleic acid of a desired target. For example, a nucleic acid molecule of the invention is preferably targeted to a highly conserved sequence region of target nucleic acids encoding VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 (specifically VEGF, VEGFR1 and/or VEGFR2 genes) such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Such nucleic acid molecules can be delivered exogenously to specific tissue or cellular targets as required. Alternatively, the nucleic acid molecules can be expressed from DNA and/or RNA vectors that are delivered to specific cells.

As used in herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism. The cell can, for example, be in vitro, e.g., in cell culture, or present in a multicellular organism, including,, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell may be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

By "VEGFR1 and/or VEGFR2 proteins" is meant, protein receptor or a mutant protein derivative thereof, having vascular endothelial growth factor receptor activity, for example, having the ability to bind vascular endothelial growth factor and/or having tyrosine kinase activity.

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By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

"Angiogenesis" refers to formation of new blood vessels which is an essential process in reproduction, development and wound repair. "Tumor angiogenesis" refers to the induction of the growth of blood vessels from surrounding tissue into a solid tumor. Tumor growth and tumor metastasis are dependent on angiogenesis (for a review see Folkman, 1985 supra; Folkman 1990 J. Natl. Cancer Inst., 82, 4; Folkman and Shing, 1992 J. Biol. Chem. 267, 10931).

Angiogenesis plays an important role in other diseases such as arthritis wherein new blood vessels have been shown to invade the joints and degrade cartilage (Folkman and Shing, supra).

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"Retinopathy" refers to inflammation of the retina and/or degenerative condition of the retina which may lead to occlusion of the retina and eventual blindness. In "diabetic retinopathy" angiogenesis causes the capillaries in the retina to invade the vitreous resulting in bleeding and blindness which is also seen in neonatal retinopathy (for a review see Folkman, 1985 supra; Folkman 1990 supra; Folkman and Shing, 1992 supra).

Nucleic acid-based inhibitors of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2, expression are useful for the prevention, treatment, and/or control of angiogenesis related disorders and conditions, including but not limited to, tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and/or endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), menopausal dysfunction, and other diseases or conditions that are related to or will respond to the levels of VEGF, VEGFR1 and/or VEGFR2 in a cell or tissue, alone or in combination with other therapies. The reduction of VEGF and/or VEGFR, such as VEGFR1 and/or VEGFR2 expression (specifically VEGF, VEGFR1 and/or VEGFR2 gene RNA levels) and thus reduction in the level of the respective protein relieves, to some degree, the symptoms of the disease or condition. Nucleic acid-based inhibitors of VEGF and/or VEGFR, such as VEGFR1 and/or VEGFR2 expression are also useful as birth control agents, for example by inhibition of ovulation or embryonic uterine implantation.

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The nucleic acid molecules of the invention can be added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection or infusion pump, with or without their incorporation in biopolymers. In preferred embodiments, the nucleic acid inhibitors comprise sequences, which are complementary to polynucleotides, for example DNA and RNA, having VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 sequence.

Triplex molecules of the invention can be provided targeted to DNA target regions, and containing the DNA equivalent of a target sequence or a sequence complementary to the specified target (substrate) sequence. Antisense molecules typically are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both.

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By "consists essentially of' is meant that the active nucleic acid molecule of the invention, for example, an enzymatic nucleic acid molecule, contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind nucleic acid such that cleavage at the target site occurs. Other sequences can be present which do not interfere with such cleavage. Thus, a core region can, for example, include one or more loop, stem-loop structure, or linker which does not prevent enzymatic activity. Thus, a particular region of a nucleic acid molecule of the invention can be such a loop, stem-loop, nucleotide linker, and/or non-nucleotide linker and can be represented generally as sequence "X". Thus, a core region may, for example, include one or more loop or stem-loop structures which do not prevent enzymatic activity. For example, a core sequence for a hammerhead enzymatic nucleic acid can comprise a conserved sequence, such as 5'-CUGAUGAG-3' and 5'-CGAA-3' connected by "X", where X is 5'-GCCGUUAGGC-3' (SEQ ID NO 5979), or any other Stem II region known in the art, or a nucleotide and/or non-nucleotide linker. Similarly, for other nucleic acid molecules of the instant invention, such as Inozyme, G-cleaver, amberzyme, zinzyme, DNAzyme, antisense, 2-5A antisense, triplex forming nucleic acid, aptamers, decoy nucleic acids, dsRNA or siRNA, other sequences or non-nucleotide linkers can be present that do not interfere with the function of the nucleic acid molecule.

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Sequence X can be a linker of ≥ 2 nucleotides in length, preferably 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 26, 30, where the nucleotides can preferably be internally base-paired to form a stem of preferably ≥ 2 base pairs. Alternatively or in addition, sequence X can be a non-nucleotide linker. In yet another embodiment, the nucleotide linker X can be a nucleic acid aptamer, such as an ATP aptamer, HIV Rev aptamer (RRE), HIV Tat aptamer (TAR) and others (for a review see Gold et al., 1995, Annu. Rev. Biochem., 64, 763; and Szostak & Ellington, 1993, in The RNA World, ed. Gesteland and Atkins, pp. 511, CSH Laboratory Press). A nucleic acid aptamer includes a nucleic acid sequence capable of interacting with a ligand. The ligand can be any natural or a synthetic molecule, including but not limited to a resin, metabolites, nucleosides, nucleotides, drugs, toxins, transition state analogs, peptides, lipids, proteins, amino acids, nucleic acid molecules, hormones, carbohydrates, receptors, cells, viruses, bacteria and others.

In yet another embodiment, the non-nucleotide linker X is as defined herein. The term "non-nucleotide" as used herein include either abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, Nucleic Acids Res. 1990, 18:6353 and Nucleic Acids Res. 1987, 15:3113; Cload and Schepartz, J. Am. Chem. Soc. 1991, 113:6324; Richardson and Schepartz, J. Am. Chem. Soc. 1991, 113:5109; Ma et al., Nucleic Acids Res. 1993, 21:2585 and Biochemistry 1993, 32:1751; Durand et al., Nucleic Acids Res. 1990, 18:6353; McCurdy et al., Nucleosides & Nucleotides 1991, 10:287; Jschke et al., Tetrahedron Lett. 1993, 34:301; Ono et al., Biochemistry 1991, 30:9914; Arnold et al., International Publication No. WO 95/06731; Dudycz et al., International Publication No. WO 95/11910 and Ferentz and Verdine, J. Am. Chem. Soc. 1991, 113:4000, all hereby incorporated by reference herein.

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A "non-nucleotide" further means any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine. Thus, in one embodiment, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

In another aspect of the invention, nucleic acid molecules that interact with target nucleic acid molecules and down-regulate VEGF and/or VEGFr, such as VEGFR1 and/or

VEGFR2 (specifically VEGF, VEGFR1 and/or VEGFR2 gene) activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Enzymatic nucleic acid molecule or antisense expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the enzymatic nucleic acid molecules or antisense are delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of enzymatic nucleic acid molecules or antisense. Such vectors can be repeatedly administered as necessary. Once expressed, the enzymatic nucleic acid molecules or antisense bind to the target nucleic acid and down-regulate its function or expression. Delivery of enzymatic nucleic acid molecule or antisense expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells explanted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell. Antisense DNA can be expressed via the use of a single stranded DNA intracellular expression vector.

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By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

By "subject" or "patient" is meant an organism, which is a donor or recipient of explanted cells, or the cells themselves. "Subject" or "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. Preferably, a subject or patient is a mammal or mammalian cells. More preferably, a subject or patient is a human or human cells.

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or in vivo where the activity is a reflection of both the catalytic activity and the stability of the nucleic acid molecules of the invention. In this invention, the product of these properties can be increased *in vivo* compared to an all RNA enzymatic nucleic acid or all DNA enzyme. In some cases, the activity or stability of the nucleic acid molecule can be decreased (i.e., less than ten-fold), but the overall activity of the nucleic acid molecule is enhanced, *in vivo*.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with the levels of VEGFR1 and/or VEGFR2, the patient can be treated, or other appropriate cells can be treated, as is evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the described molecules of the invention can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules can be used in combination with one or more known therapeutic agents to treat angiogenesis related disorders and conditions, including but not limited to tumor angiogenesis, cancers such as breast cancer, lung cancer, colorectal cancer, renal cancer, pancreatic cancer, or melanoma, or ocular indications such as diabetic retinopathy, or age related macular degeneration, and/or endometriosis, birth control, endometrial tumors, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), menopausal dysfunction, endometrial carcinoma, and/or other diseases or conditions which respond to the modulation of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 expression.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

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Figure 1 shows a secondary structure model of ANGIOZYME™ ribozyme bound to its RNA target.

Figure 2 shows a time course of inhibition of primary tumor growth following systemic administration of ANGIOZYME™ in the LLC mouse model.

Figure 3 shows inhibition of primary tumor growth following systemic administration of ANGIOZYME™ according to a certain dosing regimen in the LLC mouse model.

Figure 4 shows a dose-dependent inhibition of tumor metastases following systemic administration of ANGIOZYME™ in a mouse colorectal model.

Figure 5 is a graph showing the plasma concentration profile of ANGIOZYME™ after a single subcutaneous (SC) dose of 10, 30, 100 or 300 mg/m².

Figure 6 shows examples of chemically stabilized ribozyme motifs. HH Rz, represents hammerhead ribozyme motif (Usman et al., 1996, Curr. Op. Struct. Bio., 1, 527); NCH Rz represents the NCH ribozyme motif (Ludwig et al., International PCT Publication No. WO 98/58058 and US Patent Application Serial No. 08/878,640); G-Cleaver, represents G-cleaver ribozyme motif (Kore et al., 1998, Nucleic Acids Research 26, 4116-4120, Eckstein et

al., US 6,127,173). N or n, represent independently a nucleotide which can be same or different and have complementarity to each other; rI, represents ribo-Inosine nucleotide; arrow indicates the site of cleavage within the target. Position 4 of the HH Rz and the NCH Rz is shown as having 2'-C-allyl modification, but those skilled in the art will recognize that this position can be modified with other modifications well known in the art, so long as such modifications do not significantly inhibit the activity of the ribozyme.

Figure 7 shows an example of a Zinzyme A ribozyme motif that is chemically stabilized (see for example Beigelman et al., International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/918,728).

Figure 8 shows an example of a DNAzyme motif described by Santoro et al., 1997, PNAS, 94, 4262 and Joyce et al., US 5,807,718.

Figure 9 shows data demonstrating the inhibition of soluble VEGFR1 in a clinical study using ANGIOZYME (SEQ ID NO: 5977).

Figure 10 shows an generalized outline for the mouse model of proliferative retinopathy showing the points of ribozyme administration.

Figure 11 shows a graph demonstrating the efficacy of a VEGF-receptor-targeted enzymatic nucleic acid molecule in a mouse model of proliferative retinopathy.

Detailed Description of the Invention

Nucleic Acid Molecules and Mechanism of Action

Enzymatic Nucleic Acid: Several varieties of naturally-occurring enzymatic nucleic acids are presently known. In addition, several in vitro selection (evolution) strategies (Orgel, 1979, Proc. R. Soc. London, B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing cleavage and ligation of phosphodiester linkages (Joyce, 1989, Gene, 82, 83-87; Beaudry et al., 1992, Science 257, 635-641; Joyce, 1992, Scientific American 267, 90-97; Breaker et al., 1994, TIBTECH 12, 268; Bartel et al., 1993, Science 261:1411-1418; Szostak, 1993, TIBS 17, 89-93; Kumar et al., 1995, FASEB J., 9, 1183; Breaker, 1996, Curr. Op. Biotech., 7, 442; Santoro et al., 1997, Proc. Natl. Acad. Sci., 94, 4262; Tang et al., 1997, RNA 3, 914; Nakamaye & Eckstein, 1994, supra; Long & Uhlenbeck, 1994, supra; Ishizaka et al., 1995, supra; Vaish et al., 1997, Biochemistry 36, 6495; all of these are incorporated by reference herein). Each can catalyze a series of reactions including the hydrolysis of

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phosphodiester bonds in trans (and thus can cleave other nucleic acid molecules) under physiological conditions.

The enzymatic nature of an enzymatic nucleic acid molecule has significant advantages, one advantage being that the concentration of enzymatic nucleic acid molecule necessary to affect a therapeutic treatment is lower. This advantage reflects the ability of the enzymatic nucleic acid molecule to act enzymatically. Thus, a single enzymatic nucleic acid molecule is able to cleave many molecules of target nucleic acid. In addition, the enzymatic nucleic acid molecule is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target nucleic acid, but also on the mechanism of target nucleic acid cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a enzymatic nucleic acid molecule.

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Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. With the proper design, such enzymatic nucleic acid molecules can be targeted to RNA transcripts, and achieve efficient cleavage in vitro (Zaug et al., 324, Nature 429 1986; Uhlenbeck, 1987 Nature 328, 596; Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Dreyfus, 1988, Einstein Quart. J. Bio. Med., 6, 92; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989; Santoro et al., 1997 supra).

Because of their sequence specificity, trans-cleaving enzymatic nucleic acid molecules can be used as therapeutic agents for human disease (Usman & McSwiggen, 1995 Ann. Rep. Med. Chem. 30, 285-294; Christoffersen and Marr, 1995 J. Med. Chem. 38, 2023-2037). Enzymatic nucleic acid molecules can be designed to cleave specific nucleic acid targets within the background of cellular nucleic acid. Such a cleavage event renders the nucleic acid non-functional and abrogates protein expression from that nucleic acid. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited (Warashina et al., 1999, Chemistry and Biology, 6, 237-250).

Enzymatic nucleic acid molecules of the invention that are allosterically regulated ("allozymes") can be used to down-regulate VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2, expression. These allosteric enzymatic nucleic acids or allozymes (see for example Usman et al., US Patent Application No. 09/877,526, George et al., US Patent Nos. 5,834,186 and 5,741,679, Shih et al., US Patent No. 5,589,332, Nathan et al., US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker

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et al., International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger et al., US Patent Application Serial No. 09/205,520) are designed to respond to a signaling agent, for example, mutant VEGFR1 and/or VEGFR2 protein, wild-type VEGFR1 and/or VEGFR2 protein, mutant VEGFR1 and/or VEGFR2 RNA, wild-type VEGFR1 and/or VEGFR2 RNA, other proteins and/or RNAs involved in VEGF signal transduction, compounds, metals, polymers, molecules and/or drugs that are targeted to VEGFR1 and/or VEGFR2 expression, which in turn modulates the activity of the enzymatic nucleic acid molecule. In response to interaction with a predetermined signaling agent, the activity of the allosteric enzymatic nucleic acid is activated or inhibited such that the expression of a particular target is selectively down-regulated. The target can comprise wild-type VEGFR1 and/or VEGFR2, mutant VEGFR1 and/or VEGFR2, and/or a predetermined component of the VEGF signal transduction pathway. In a specific example, allosteric enzymatic nucleic acid molecules that are activated by interaction with a RNA encoding VEGF protein are used as therapeutic agents in vivo. The presence of RNA encoding the VEGF protein activates the allosteric enzymatic nucleic acid molecule that subsequently cleaves the RNA encoding a VEGFR1 and/or VEGFR2 protein resulting in the inhibition of VEGFR1 and/or VEGFR2 protein expression.

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In another non-limiting example, an allozyme can be activated by a VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 protein, peptide, or mutant polypeptide that causes the allozyme to inhibit the expression of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 genes, by, for example, cleaving RNA encoded by VEGF, VEGFR1 and/or VEGFR2 gene. In this non-limiting example, the allozyme acts as a decoy to inhibit the function of VEGF, VEGFR1 and/or VEGFR2 and also inhibit the expression of VEGF, VEGFR1 and/or VEGFR2 once activated by the VEGF, VEGFR1 and/or VEGFR2 protein.

Antisense: Antisense molecules can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides and primarily function by specifically binding to matching sequences resulting in inhibition of peptide synthesis (Wu-Pong, Nov 1994, BioPharm, 20-33). The antisense oligonucleotide binds to target RNA by Watson Crick base-pairing and blocks gene expression by preventing ribosomal translation of the bound sequences either by steric blocking or by activating RNase H enzyme. Antisense molecules can also alter protein synthesis by interfering with RNA processing or transport from the nucleus into the cytoplasm (Mukhopadhyay & Roth, 1996, Crit. Rev. in Oncogenesis 7, 151-190).

In addition, binding of single stranded DNA to RNA can result in nuclease degradation of the heteroduplex (Wu-Pong, supra; Crooke, supra). To date, the only backbone modified

DNA chemistry which act as substrates for RNase H are phosphorothioates, phosphorodithioates, and borontrifluoridates. Recently it has been reported that 2'-arabino and 2'-fluoro arabino- containing oligos can also activate RNase H activity.

A number of antisense molecules have been described that utilize novel configurations of chemically modified nucleotides, secondary structure, and/or RNase H substrate domains (Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., International PCT Publication No. WO 99/54459; Hartmann et al., USSN 60/101,174 which was filed on September 21, 1998) all of these are incorporated by reference herein in their entirety.

In addition, antisense deoxyoligoribonucleotides can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. Antisense DNA can be expressed via the use of a single stranded DNA intracellular expression vector or equivalents and variations thereof.

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Triplex Forming Oligonucleotides (TFO): Single stranded DNA can be designed to bind to genomic DNA in a sequence specific manner. TFOs are comprised of pyrimidine-rich oligonucleotides which bind DNA helices through Hoogsteen Base-pairing (Wu-Pong, supra). The resulting triple helix composed of the DNA sense, DNA antisense, and TFO disrupts RNA synthesis by RNA polymerase. The TFO mechanism can result in gene expression or cell death since binding can be irreversible (Mukhopadhyay & Roth, supra).

2-5A Antisense Chimera: The 2-5A system is an interferon mediated mechanism for RNA degradation found in higher vertebrates (Mitra et al., 1996, Proc Nat Acad Sci USA 93, 6780-6785). Two types of enzymes, 2-5A synthetase and RNase L, are required for RNA cleavage. The 2-5A synthetases require double stranded RNA to form 2'-5' oligoadenylates (2-5A). 2-5A then acts as an allosteric effector for utilizing RNase L which has the ability to cleave single stranded RNA. The ability to form 2-5A structures with double stranded RNA makes this system particularly useful for inhibition of viral replication.

(2'-5') oligoadenylate structures can be covalently linked to antisense molecules to form chimeric oligonucleotides capable of RNA cleavage (Torrence, *supra*). These molecules putatively bind and activate a 2-5A dependent RNase, the oligonucleotide/enzyme complex then binds to a target RNA molecule which can then be cleaved by the RNase enzyme.

RNAi: Double-stranded RNAs can suppress expression of homologous genes through an evolutionarily conserved process named RNA interference (RNAi) or post-transcriptional gene silencing (PTGS). One mechanism underlying silencing is the degradation of target mRNAs by an RNP complex, which contains short interfering RNAs (siRNAs) as guides to substrate selection. Short interfering RNAs are typically 21 to 23 nucleotides in length. A bidentate nuclease called Dicer has been implicated as the protein responsible for siRNA production. For example, a double-stranded RNA (dsRNA) matching a gene sequence is synthesized in vitro and introduced into a cell. The dsRNA feeds into a biological pathway and is broken into short pieces of short interfering (si) RNAs. With the help of cellular enzymes such as Dicer, the siRNA triggers the degradation of the messenger RNA that matches its sequence (see for example Tuschl et al., International PCT Publication No. WO 01/75164; Bass, 2001, Nature, 411, 428-429; Elbashir et al., 2001, Nature, 411, 494-498; and Kreutzer et al., International PCT Publication No. WO 00/44895).

Target sites

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Targets for useful nucleic acid molecules of the invention, such as enzymatic nucleic acid molecules, dsRNA, and antisense nucleic acids can be determined as disclosed in Draper et al., WO 93/23569; Sullivan et al., WO 93/23057; Thompson et al., WO 94/02595; Draper et al., WO 95/04818; McSwiggen et al., US Patent No. 5,525,468, and hereby incorporated by reference herein in totality. Other examples include the following PCT applications, which concern inactivation of expression of disease-related genes: WO 95/23225, WO 95/13380, WO 94/02595, incorporated by reference herein. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Enzymatic nucleic acid molecules and antisense to such targets are designed as described in those applications and synthesized to be tested in vitro and in vivo, as also described. The sequences of human VEGF, VEGFR1 and/or VEGFR2 RNAs are screened for optimal nucleic acid target sites using a computer-folding algorithm. Potential nucleic acid binding/cleavage sites are identified. While human sequences can be screened and nucleic acid molecules thereafter designed, as discussed in Stinchcomb et al., WO 95/23225, mouse targeted enzymatic nucleic acid molecules can be useful to test efficacy of action of the nucleic acid molecule prior to testing in humans.

Nucleic acid molecule binding/cleavage sites are identified, for example enzymatic nucleic acid, antisense, and dsRNA mediated binding sites are chosen. For enzymatic nucleic acid molecules of the invention, the nucleic acid molecules are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706) to assess whether

the sequences fold into the appropriate secondary structure. Those nucleic acid molecules with unfavorable intramolecular interactions such as between the binding arms and the catalytic core can be eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity.

Nucleic acids, such as antisense, RNAi, and/or enzymatic nucleic acid molecule binding/cleavage sites are identified and are designed to anneal to various sites in the nucleic acid target. The binding arms of enzymatic nucleic acid molecules of the invention are complementary to the target site sequences described above. Antisense and RNAi sequences are designed to have partial or complete complementarity to the nucleic acid target. The nucleic acid molecules can be chemically synthesized. The method of synthesis used follows the procedure for normal DNA/RNA synthesis as described below and in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845; Scaringe et al., 1990 Nucleic Acids Res., 18, 5433; and Wincott et al., 1995 Nucleic Acids Res. 23, 2677-2684; Caruthers et al., 1992, Methods in Enzymology 211,3-19.

Synthesis of Nucleic acid Molecules 15

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Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small refers to nucleic acid motifs less than about 100 nucleotides in length, preferably less than about 80 nucleotides in length, and more preferably less than about 50 nucleotides in length; e.g., antisense oligonucleotides, enzymatic nucleic acids, aptamers, allozymes, decoys, siRNA etc.) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

DNA Oligonucleotides are synthesized using protocols known in the art as described in Caruthers et al., 1992, Methods in Enzymology 211, 3-19, Thompson et al., International PCT Publication No. WO 99/54459, Wincott et al., 1995, Nucleic Acids Res. 23, 2677-2684, Wincott et al., 1997, Methods Mol. Bio., 74, 59, Brennan et al., 1998, Biotechnol Bioeng., 61, 33-45, and Brennan, US patent No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting 30 and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 µmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides. Table II

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outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'hydroxyl. A 22-fold excess (40 μ L of 0.11 M = 4.4 μ mol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μ L of 0.25 M = 10 μ mol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I₂, 49 mM pyridine, 9% water in THF (PERSEPTIVETM). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

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Deprotection of the DNA polynucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA oligonucleotides including certain nucleic acid molecules of the invention follows the procedure as described in Usman et al., 1987, J. Am. Chem. Soc., 109, 7845; Scaringe et al., 1990, Nucleic Acids Res., 18, 5433; and Wincott et al., 1995, Nucleic Acids Res. 23, 2677-2684 Wincott et al., 1997, Methods Mol. Bio., 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 µmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table II outlines the amounts and the

contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 µmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μ L of 0.11 M = 13.2 µmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μ L of 0.25 M = 30 μ mol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 µL of a solution of 1.5 mL N-methylpyrrolidinone, 750 µL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH₄HCO₃.

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Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to r.t. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at -20 °C and then quenched with 1.5 M NH₄HCO₃.

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For purification of the trityl-on oligomers, the quenched NH₄HCO₃ solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

Inactive hammerhead ribozymes or binding attenuated control (BAC) oligonucleotides) are synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel, K. J., et al., 1992, Nucleic Acids Res., 20, 3252). Similarly, one or more nucleotide substitutions can be introduced in other enzymatic nucleic acid molecules to inactivate the molecule and such molecules can serve as a negative control.

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The average stepwise coupling yields are typically >98% (Wincott et al., 1995 Nucleic Acids Res. 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96 well format, all that is important is the ratio of chemicals used in the reaction.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example by ligation (Moore et al., 1992, Science 256, 9923; Draper et al., International PCT publication No. WO 93/23569; Shabarova et al., 1991, Nucleic Acids Research 19, 4247; Bellon et al., 1997, Nucleosides & Nucleotides, 16, 951; Bellon et al., 1997, Bioconjugate Chem. 8, 204).

Preferably, the nucleic acid molecules of the present invention are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, TIBS 17, 34; Usman et al., 1994, Nucleic Acids Symp. Ser. 31, 163). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott et al., Supra, the totality of which is hereby incorporated herein by reference) and are re-suspended in water.

Optimizing Activity of the nucleic acid molecule of the invention.

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases can increase their potency (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991, Science 253, 314; Usman and Cedergren, 1992, Trends

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in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; Gold et al., US 6,300,074; and Burgin et al., supra; all of which are incorporated by reference herein). Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired. (All these publications are hereby incorporated by reference herein).

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There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, TIBS. 17, 34; Usman et al., 1994, Nucleic Acids Symp. Ser. 31, 163; Burgin et al., 1996, Biochemistry, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein et al., International Publication PCT No. WO 92/07065; Perrault et al. Nature, 1990, 344, 565-568; Pieken et al. Science, 1991, 253, 314-317; Usman and Cedergren, Trends in Biochem. Sci., 1992, 17, 334-339; Usman et al. International Publication PCT No. WO 93/15187; Sproat, US Patent No. 5,334,711 and Beigelman et al., 1995, J. Biol. Chem., 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., US Patent No. 5,716,824; Usman et al., US patent No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., USSN 60/082,404 which was filed on April 20, 1998; Karpeisky et al., 1998, Tetrahedron Lett., 39, 1131; Earnshaw and Gait, 1998, Biopolymers (Nucleic acid Sciences), 48, 39-55; Verma and Eckstein, 1998, Annu. Rev. Biochem., 67, 99-134; and Burlina et al., 1997, Bioorg. Med. Chem., 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without inhibiting catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, too many of these modifications can cause some toxicity. Therefore when designing nucleic acid molecules the amount of these internucleotide linkages should be minimized.

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The reduction in the concentration of these linkages should lower toxicity resulting in increased efficacy and higher specificity of these molecules.

Nucleic acid molecules having chemical modifications that maintain or enhance activity are provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or in vivo the activity may not be significantly lowered. Therapeutic nucleic acid molecules delivered exogenously are optimally stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, nucleic acid molecules must be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of RNA and DNA (Wincott et al., 1995 Nucleic Acids Res. 23, 2677; Caruthers et al., 1992, Methods in Enzymology 211,3-19 (incorporated by reference herein) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

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In one embodiment, nucleic acid molecules of the invention include one or more G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, J. Am. Chem. Soc., 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets. In another embodiment, nucleic acid molecules of the invention include one or more LNA "locked nucleic acid" nucleotides such as a 2', 4'-C mythylene bicyclo nucleotide (see for example Wengel et al., International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of nucleic acid molecules targeting VEGF receptors such as VEGFR1 and/or VEGFR2. Such conjugates and/or complexes can be used to facilitate delivery of molecules into a biological system, such as cells. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including but not limited to small

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molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, US 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

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The term "biodegradable nucleic acid linker molecule" as used herein, refers to a nucleic acid molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule. The stability of the biodegradable nucleic acid linker molecule can be modulated by using various combinations of ribonucleotides, deoxyribonucleotides, and chemically modified nucleotides, for example, 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siRNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active

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molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (e.g., enzymatic nucleic acid molecules and antisense nucleic acid molecules) delivered exogenously are optimally stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. These nucleic acid molecules should be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

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In another embodiment, nucleic acid catalysts having chemical modifications that maintain or enhance enzymatic activity are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or in vivo the activity of the nucleic acid may not be significantly lowered. As exemplified herein such enzymatic nucleic acids are useful in a cell and/or in vivo even if activity over all is reduced 10 fold (Burgin et al., 1996, Biochemistry, 35, 14090). Such enzymatic nucleic acids herein are said to "maintain" the enzymatic activity of an all RNA ribozyme or all DNA DNAzyme.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'- cap structure.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see for example Wincott et al., WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both terminus. In non-limiting examples, the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alphanucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-

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dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott et al., International PCT publication No. WO 97/26270, incorporated by reference herein).

In another embodiment the 3'-cap includes, for example 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, Tetrahedron 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =0, =S, NO₂ or N(CH3)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =0, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least

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one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =0, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated p electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which can be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

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By "nucleotide" is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, for example, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g.,

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5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, quesosine, 2wybutoxosine, 4-acetylcytidine, wybutosine, 4-thiouridine, thiouridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-(carboxyhydroxymethyl)uridine, 1-methyladenosine, beta-D-galactosylqueosine, carboxymethylaminomethyluridine, 3-methylcytidine, 2-methyladenosine, 2-2,2-dimethylguanosine, methylinosine, 5-methoxyaminomethyl-2-7-methylguanosine, N6-methyladenosine, methylguanosine, 5-methylcarbonylmethyluridine, 5-methylaminomethyluridine, thiouridine, methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-Dmannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin et al., 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

By "nucleoside" is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a sugar. Nucleosides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleoside sugar moiety. Nucleosides generally comprise a base and sugar group. The nucleosides can be unmodified or modified at the sugar, and/or base moiety, (also referred to interchangeably as nucleoside analogs, modified nucleosides, non-natural nucleosides, nonstandard nucleosides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183. Some of the nonlimiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutososine, 5'-carboxymethylaminomethyl-2-5-(carboxyhydroxymethyl)uridine, 4-acetylcytidine, beta-D-galactosylqueosine, 5-carboxymethylaminomethyluridine, 1thiouridine, 3-methylcytidine, 2-2,2-dimethylguanosine, 1-methylinosine, methyladenosine, N6-methyladenosine, 7-methylguanosine, 5-2-methylguanosine, methyladenosine,

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methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin et al., 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleoside bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases can be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

In one embodiment, the invention features modified enzymatic nucleic acid molecules with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications see Hunziker and Leumann, 1995, Nucleic Acid Analogues: Synthesis and Properties, in Modern Synthetic Methods, VCH, 331-417, and Mesmaeker et al., 1994, Novel Backbone Replacements for Oligonucleotides, in Carbohydrate Modifications in Antisense Research, ACS, 24-39. These references are hereby incorporated by reference herein.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, for example a 3',3'-linked or 5',5'-linked deoxyabasic ribose derivative (for more details see Wincott et al., International PCT publication No. WO 97/26270).

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of β -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH₂ or 2'-O- NH₂, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein et al., U.S. Patent 5,672,695 and Matulic-Adamic et al., WO 98/28317, respectively, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid (e.g., antisense and ribozyme) structure can be made to enhance the utility of these molecules. For example, such modifications can enhance

shelf-life, half-life in vitro, stability, and ease of introduction of such oligonucleotides to the target site, including, e.g., enhancing penetration of cellular membranes and conferring the ability to recognize and bind to targeted cells.

Use of the nucleic acid-based molecules of the invention can lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of enzymatic nucleic acid molecules (including different enzymatic nucleic acid molecule motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules can also include combinations of different types of nucleic acid molecules. Therapies can be devised which include a mixture of enzymatic nucleic acid molecules (including different enzymatic nucleic acid molecule motifs), allozymes, antisense, dsRNA, aptamers, and/or 2-5A chimera molecules to one or more targets to alleviate symptoms of a disease.

15 Administration of Nucleic Acid Molecules

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Methods for the delivery of nucleic acid molecules are described in Akhtar et al., 1992, Trends Cell Bio., 2, 139; and Delivery Strategies for Antisense Oligonucleotide Therapeutics, ed. Akhtar, 1995 which are both incorporated herein by reference. Sullivan et al., PCT WO 94/02595, further describes the general methods for delivery of enzymatic RNA molecules. These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Other routes of delivery include, but are not limited to oral (tablet or pill form) and/or intrathecal delivery (Gold, 1997, Neuroscience, 76, 1153-1158). Other approaches include the use of various transport and carrier systems, for example though the use of conjugates and biodegradable polymers. For a comprehensive review on drug delivery strategies including CNS delivery, see Ho et al., 1999, Curr. Opin. Mol. Ther., 1, 336-343 and Jain, Drug Delivery Systems: Technologies and Commercial Opportunities, Decision Resources, 1998 and Groothuis et al., 1997, J. Neuro Virol., 3, 387-400. More detailed descriptions of nucleic acid delivery and administration are provided in Sullivan et al., supra, Draper et al., PCT

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WO93/23569, Beigelman et al., PCT WO99/05094, and Klimuk et al., PCT WO99/04819 all of which have been incorporated by reference herein.

The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

The polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced into a patient by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and the other compositions known in the art.

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The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, e.g., systemic administration, into a cell or patient, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged polymers, e.g., nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can

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potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cells implicated in endometriosis, birth control, endometrial tumors, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), menopausal dysfunction, and endometrial carcinoma.

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By pharmaceutically acceptable formulation is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: PEG conjugated nucleic acids, phospholipid conjugated nucleic acids, nucleic acids containing lipophilic moieties, phosphorothioates, P-glycoprotein inhibitors (such as Pluronic P85) which can enhance entry of drugs into various tissues, for example the CNS (Jolliet-Riant and Tillement, 1999, Fundam. Clin. Pharmacol., 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after implantation (Emerich, DF et al, 1999, Cell Transplant, 8, 47-58) Alkermes, Inc. Cambridge, MA; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (Prog Neuropsychopharmacol Biol Psychiatry, 23, 941-949, 1999). Other non-limiting examples of delivery strategies, including CNS delivery of the nucleic acid molecules of the instant invention include material described in Boado et al., 1998, J. Pharm. Sci., 87, 1308-1315; Tyler et al., 1999, FEBS Lett., 421, 280-284; Pardridge et al., 1995, PNAS USA., 92, 5592-5596; Boado, 1995, Adv. Drug Delivery Rev., 15, 73-107; Aldrian-Herrada et al., 1998, Nucleic Acids Res., 26, 4910-4916; and Tyler et al., 1999, PNAS USA., 96, 7053-7058. All these references are hereby incorporated herein by reference.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). Nucleic acid molecules of the invention can also comprise covalently attached PEG molecules of various molecular weights. These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. Chem. Rev. 1995, 95, 2601-2627; Ishiwata et al., Chem.

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Pharm. Bull. 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., Science 1995, 267, 1275-1276; Oku et al., 1995, Biochim. Biophys. Acta, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et al., J. Biol. Chem. 1995, 42, 24864-24870; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10392; all of which are incorporated by reference herein). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen. All of these references are incorporated by reference herein.

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of phydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or

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infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

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Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters

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derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

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Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and

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isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The nucleic acid molecules of the invention can also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

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Alternatively, certain of the nucleic acid molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, Science, 229, 345; McGarry and Lindquist, 1986, Proc. Natl. Acad. Sci., USA 83, 399; Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992, Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992, J. Virol., 66, 1432-41; Weerasinghe et al., 1991, J. Virol., 65, 5531-4; Ojwang et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992, Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science, 247, 1222-1225; Thompson et al., 1995, Nucleic Acids Res., 23, 2259; Good et al., 1997, Gene Therapy, 4, 45; all of these references are hereby incorporated in their totalities by reference herein). Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the 10 appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992, Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993, Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994, J. Biol. Chem., 269, 25856; all of these references are hereby incorporated in their totalities by reference herein). Gene therapy approaches specific to the CNS are described by Blesch et al., 2000, Drug News Perspect., 13, 269-280; Peterson et al., 2000, Cent. Nerv. Syst. Dis., 485-508; Peel and Klein, 2000, J. Neurosci. Methods, 98, 95-104; Hagihara et al., 2000, Gene Ther., 7, 759-763; and Herrlinger et al., 2000, Methods Mol. Med., 35, 287-312. AAV-mediated delivery of nucleic acid to cells 20 of the nervous system is further described by Kaplitt et al., US 6,180,613.

In another aspect of the invention, RNA molecules of the present invention are preferably expressed from transcription units (see for example Couture et al., 1996, TIG., 12, 510) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the nucleic acid molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA. Delivery of nucleic acid molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review see Couture et al., 1996, TIG., 12, 510).

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In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules of the instant invention. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operably linked in a manner which allows expression of that nucleic acid molecule.

In another aspect the invention features an expression vector comprising: a) a transcription initiation region (e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, Proc. Natl. Acad. Sci. US A, 87, 6743-7; Gao and Huang 1993, Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993, Methods Enzymol., 217, 47-66; Zhou et al., 1990, Mol. Cell. Biol., 10, 4529-37). All of these references are incorporated by reference herein. Several investigators have demonstrated that nucleic acid molecules, such as ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992, Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992, Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992, Nucleic Acids Res., 20, 4581-9; Yu et al., 1993, Proc. Natl. Acad. Sci. USA, 90, 6340-4; L'Huillier et al., 1992, EMBO J., 11, 4411-8; Lisziewicz et al., 1993, Proc. Natl. Acad. Sci. U. S. A, 90, 8000-4; Thompson et al., 1995, Nucleic Acids Res., 23, 2259; Sullenger & Cech, 1993, Science, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as ribozymes in cells (Thompson et al., supra; Couture and Stinchcomb, 1996, supra; Noonberg et al., 1994, Nucleic Acid Res., 22, 2830; Noonberg et al., US Patent No. 5,624,803; Good et al., 1997, Gene Ther., 4, 45; Beigelman et al., International PCT Publication No. WO

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96/18736; all of these publications are incorporated by reference herein. The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, supra).

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In another aspect the invention features an expression vector comprising nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention, in a manner which allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; d) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. In yet another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

Flt-1 (VEGFR1), KDR (VEGFR2) and/or flk-1 are attractive nucleic acid-based therapeutic targets by several criteria. The interaction between VEGF and VEGF-R is well-established. Efficacy can be tested in well-defined and predictive animal models. Finally, the disease conditions are serious and current therapies are inadequate. Whereas protein-based

therapies are designed to affect VEGF activity, nucleic acid-based therapy based on the molecules and methods described herein provides a direct and elegant approach to directly modulate flt-1, KDR and/or flk-1 expression.

Because VEGFR1 and VEGFR2 mRNAs are highly homologous in certain regions, some nucleic acid target sites are also homologous. In this case, a single nucleic acid molecule of the invention can target both VEGFR1 and VEGFR2 mRNAs. At partially homologous sites, a single nucleic acid molecule can sometimes be designed to accommodate a site on both mRNAs by including G/U base pairing. For example, if there is a G present in a enzymatic nucleic acid target site in VEGFR1 mRNA at the same position there is an A in the VEGFR2 enzymatic nucleic acid target site, the enzymatic nucleic acid can be synthesized with a U at the complementary position and it will bind both to sites. The advantage of one enzymatic nucleic acid that targets both VEGFR1 and VEGFR2 mRNAs is clear, especially in cases where both VEGF receptors may contribute to the progression of angiogenesis in the disease state.

15 <u>Examples</u>

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The following are non-limiting examples showing the selection, isolation, synthesis and activity of exemplary nucleic acids of the instant invention.

The following examples demonstrate the selection and design of antisense, aptamer, dsRNA, allozyme, hammerhead, DNAzyme, NCH, Amberzyme, Zinzyme, or G-Cleaver ribozyme molecules and binding/cleavage sites within VEGF, VEGFR1 and/or VEGFR2 RNA.

Example 1: Enzymatic nucleic acid-mediated inhibition of angiogenesis in vivo

The study described below was performed to assess the anti-angiogenic activity of hammerhead ribozymes targeted against flt-1 4229 site (SED ID NO: 5977) in the rat cornea model of VEGF induced angiogenesis (see above). These ribozymes have either active or inactive catalytic core and either bind and cleave or just bind to VEGF-R mRNA of the flt-1 subtype. The active ribozymes, that are able to bind and cleave the target RNA, have been shown to inhibit (125I-labeled) VEGF binding in cultured endothelial cells and produce a dose-dependent decrease in VEGF induced endothelial cell proliferation in these cells. The catalytically inactive forms of these ribozymes, which can only bind to the RNA but cannot catalyze RNA cleavage, failed to inhibit VEGF binding and failed to decrease VEGF induced endothelial cell proliferation. The ribozymes and VEGF were co-delivered using the filter

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disk method: Nitrocellulose filter disks (Millipore®) of 0.057 diameter were immersed in appropriate solutions and were surgically implanted in rat cornea as described by Pandey et al., supra. This delivery method has been shown to deliver rhodamine-labeled free ribozyme to scleral cells and, in all likelihood cells of the pericorneal vascular plexus. Since the active ribozymes show cell culture efficacy and can be delivered to the target site using the disk method, it is essential that these ribozymes be assessed for in vivo anti-angiogenic activity.

The stimulus for angiogenesis in this study was the treatment of the filter disk with 30 µM VEGF which is implanted within the cornea's stroma. This dose yields reproducible neovascularization stemming from the pericorneal vascular plexus growing toward the disk in a dose-response study 5 days following implant. Filter disks treated only with the vehicle for VEGF show no angiogenic response. The ribozymes were co-adminstered with VEGF on a disk in two different ribozyme concentrations. One concern with the simultaneous administration is that the ribozymes will not be able to inhibit angiogenesis since VEGF receptors can be stimulated. However, we have observed that in low VEGF doses, the neovascular response reverts to normal suggesting that the VEGF stimulus is essential for maintaining the angiogenic response. Blocking the production of VEGF receptors using simultaneous administration of anti-VEGF-R mRNA ribozymes could attenuate the normal neovascularization induced by the filter disk treated with VEGF.

Materials and Methods:

20 1. Stock hammerhead ribozyme solutions:

a. flt-1 4229 (786 µM)- Active

b. flt-1 4229 (736 μM)— Inactive

2. Experimental solutions/groups:

Group 1 Solution 1 Control VEGF solution: 30 µM in 82mM Tris base

25 Group 2 Solution 2 flt-1 4229 (1 $\mu g/\mu L$) in 30 μM VEGF/82 mM Tris base

Group 3 Solution 3 flt-1 4229 (10 µg/µL) in 30 µM VEGF/82 mM Tris base

Group 4 Solution 4 No VEGF, flt-1 4229 (10 µg/µL) in 82 mM Tris base

Group 5 Solution 5 No VEGF, No ribozyme in 82 mM Tris base

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10 eyes per group, 5 animals (Since they have similar molecular weights, the molar concentrations should be essentially similar).

Each solution (VEGF and RIBOZYMES) were prepared as a 2X solution for 1:1 mixing for final concentrations above, with the exception of solution 1 in which VEGF was 2X and diluted with ribozyme diluent (sterile water).

3. <u>VEGF Solutions</u>

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The 2X VEGF solution (60 μ M) was prepared from a stock of 0.82 μ g/ μ L in 50 mM Tris base. 200 μ L of VEGF stock was concentrated by speed vac to a final volume of 60.8 μ L, for a final concentration of 2.7 μ g/ μ L or 60 μ M. Six 10 μ L aliquots was prepared for daily mixing. 2X solutions for VEGF and Ribozyme was stored at 4°C until the day of the surgery. Solutions were mixed for each day of surgery. Original 2X solutions was prepared on the day before the first day of the surgery.

4. <u>Surgical Solutions:</u>

Anesthesia:

stock ketamine hydrochloride 100 mg/mL

stock xylazine hydrochloride 20 mg/mL

stock acepromazine 10 mg/mL

<u>Final anesthesia solution</u>: 50 mg/mL ketamine, 10 mg/mL xylazine, and 0.5 mg/mL acepromazine

20 5% povidone iodine for opthalmic surgical wash

2% lidocaine (sterile) for opthalmic administration (2 drops per eye)

sterile 0.9% NaCl for opthalmic irrigation

5. Surgical Methods:

Standard surgical procedure as described in Pandey et al., supra. Filter disks were incubated in 1 µL of each solution for approximately 30 minutes prior to implantation.

6. Experimental Protocol:

The animal cornea were treated with the treatment groups as described above. Animals were allowed to recover for 5 days after treatment with daily observation (scoring 0 - 3). On the fifth day animals were euthanized and digital images of each eye was obtained for quantitaion using Image Pro Plus. Quantitated neovascular surface area were analyzed by ANOVA followed by two post-hoc tests including Dunnets and Tukey-Kramer tests for significance at the 95% confidence level. Dunnets provide information on the significance between the differences within the means of treatments vs. controls while Tukey-Kramer provide information on the significance of differences within the means of each group.

The flt-1 4229 (SEQ ID NO: 5977) active hammerhead ribozyme at both concentrations was effective at inhibiting angiogenesis while the inactive ribozyme did not show any significant reduction in angiogenesis. A statistically signifiant reduction in neovascular surface area was observed only with active ribozymes. This result clearly shows that the ribozymes are capable of significantly inhibiting angiogenesis in vivo. Specifically, given ribozyme mechanism of action, the observed inhibition is by the binding and cleavage of target RNA by ribozymes.

Example 2: Bioactivity of anti-angiogenesis ribozymes targeting flt-1 and kdr RNA

MATERIALS AND METHODS

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Ribozymes: Hammerhead ribozymes and controls designed to have attenuated activity (attenuated controls) were synthesized and purified as previously described above. The attenuated ribozyme controls maintain the binding arm sequence of the parent ribozyme and thus are still capable of binding to the mRNA target. However, they have two nucleotide changes in the core sequence that substantially reduce their ability to carry out the cleavage reaction. Ribozymes were designed to target Flt-1 or KDR mRNA sites conserved in human, mouse, and rat. In general, ribozymes with binding arms of seven nucleotides were designed and tested. If, however, only six nucleotides surrounding the cleavage site were conserved in all three species, six nucleotide binding arms were used. Data are presented herein for 2'-NH₂ uridine modified ribozymes in cell proliferation studies and for 2'-C-allyl uridine modified ribozymes in RNAse protection, in vitro cleavage and corneal studies.

In vitro ribozyme cleavage assays: In vitro RNA cleavage rates on a 15 nucleotide synthetic RNA substrate were measured as previously described above.

Cell culture: Human dermal microvascular endothelial cells (HMVEC-d, Clonetics Corp.) were maintained at 37°C in flasks or plates coated with 1.5% porcine skin gelatin (300)

bloom, Sigma) in Growth medium (Clonetics Corp.) supplemented with 10-20% fetal bovine serum (FBS, Hyclone). Cells were grown to confluency and used up to the seventh passage. Stimulation medium consisted of 50% Sigma 99 media and 50% RPMI 1640 with L-glutamine and additional supplementation with 10 μg/mL Insulin-Transferrin-Selenium (Gibco BRL) and 10% FBS. Cell growth was stimulated by incubation in Stimulation medium supplemented with 20 ng/mL of either VEGF₁₆₅ or bFGF. VEGF₁₆₅ (165 amino acids) was selected for cell culture and animal studies because it is the predominant form of the four native forms of VEGF generated by alternative mRNA splicing. Cell culture assays were carried out in triplicate.

Ribozyme and ribozyme/LIPOFECTAMINETM formulations:

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Cell culture: Ribozymes or attenuated controls (50-200 nM) were formulated for cell culture studies and used immediately. Formulations were carried out with LIPOFECTAMINE™ (Gibco BRL) at a 3:1 lipid to phosphate charge ratio in serum-free medium (OPTI-MEM™, Gibco BRL) by mixing for 20 minutes at room temperature. For example, a 3:1 lipid to phosphate charge ratio was established by complexing 200 nM ribozyme with 10.8 μg/μL LIPOFECTAMINE™ (13.5 μM DOSPA).

In vivo: For corneal studies, lyophilized ribozyme or attenuated controls were resuspended in sterile water at a final stock concentration of 170 μ g/ μ L (highest dose). Lower doses (1.7-50 μ g/ μ L) were prepared by serial dilution in sterile water.

Proliferation assay: HMVEC-d were seeded (5 x 10³ cells/well) in 48-well plates (Costar) and incubated 24-30 hours in Growth medium at 37°C. After removal of the Growth medium, cells were treated with 50-200 nM LIPOFECTAMINETM complexes of ribozyme or attenuated controls for 2 hours in OPTI-MEMTM. The ribozyme/control-containing medium was removed and the cells were washed extensively in 1X PBS. The medium was then replaced with Stimulation medium or Stimulation medium supplemented with 20 ng/mL VEGF₁₆₅ or bFGF. After 48 hours, the cell number was determined using a CoulterTM cell counter. Data are presented as cell number per well following 48 hours of VEGF stimulation.

RNAse protection assay: HMVEC-d were seeded (2 x 10⁵ cells/well) in 6-well plates (Costar) and allowed to grow 32-36 hours in Growth medium at 37°C. Cells were treated with LIPOFECTAMINETM complexes containing 200 nM ribozyme or attenuated control for 2 h as described under "Proliferation Assay" and then incubated in Growth medium containing 20 ng/mL VEGF₁₆₅ for 24 hours. Cells were harvested and an RNAse protection assay was carried out using the Ambion Direct Protect kit and protocol with the exception that 50 mM

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EDTA was added to the lysis buffer to eliminate the possibility of ribozyme cleavage during sample preparation. Antisense RNA probes targeting portions of Flt-1 and KDR were prepared by transcription in the presence of [32 P]-UTP. Samples were analyzed on polyacrylamide gels and the level of protected RNA fragments was quantified using a Molecular Dynamics PhosphorImager. The levels of Flt-1 and KDR were normalized to the level of cyclophilin (human cyclophilin probe template, Ambion) in each sample. The coefficient of variation for cyclophilin levels was 11% [265940 cpm \pm 29386 (SD)] for all conditions tested here (i.e. in the presence of either active ribozymes or attenuated controls). Thus, cyclophilin is useful as an internal standard in these studies.

Rat corneal pocket assay of VEGF-induced angiogenesis:

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Animal guidelines and anesthesia. Animal housing and experimentation adhered to standards outlined in the 1996 Guide for the Care and Use of Laboratory Animals (National Research Council). Male Sprague Dawley rats (250-300 g) were anesthetized with ketamine (50 mg/kg), xylazine (10 mg/kg), and acepromazine (0.5 mg/kg) administered intramuscularly (im). The level of anesthesia was monitored every 2-3 min by applying hind limb paw pressure and examining for limb withdrawal. Atropine (0.4 mg/kg, im) was also administered to prevent potential corneal reflex-induced bradycardia.

Preparation of VEGF soaked disk. For corneal implantation, 0.57 mm diameter nitrocellulose disks, prepared from 0.45 μm pore diameter nitrocellulose filter membranes (Millipore Corporation), were soaked for 30 min in 1 μL of 30 μM VEGF₁₆₅ in 82 mM Tris HCl (pH 6.9) in covered petri dishes on ice.

Corneal surgery. The rat corneal model used in this study was a modified from Koch et al. Supra and Pandey et al., supra. Briefly, corneas were irrigated with 0.5% povidone iodine solution followed by normal saline and two drops of 2% lidocaine. Under a dissecting microscope (Leica MZ-6), a stromal pocket was created and a presoaked filter disk (see above) was inserted into the pocket such that its edge was 1 mm from the corneal limbus.

Intraconjunctival injection of test solutions. Immediately after disk insertion, the tip of a 40-50 µm OD injector (constructed in our laboratory) was inserted within the conjunctival tissue 1 mm away from the edge of the corneal limbus that was directly adjacent to the VEGF-soaked filter disk. Six hundred nanoliters of test solution (ribozyme, attenuated control or sterile water vehicle) were dispensed at a rate of 1.2 µL/min using a syringe pump (Kd Scientific). The injector was then removed, serially rinsed in 70% ethanol and sterile water and immersed in sterile water between each injection. Once the test solution was injected,

closure of the eyelid was maintained using microaneurism clips until the animal began to recover gross motor activity. Following treatment, animals were warmed on a heating pad at 37°C.

Animal treatment groups/experimental protocol. Ribozymes targeting Flt-1 site 4229 (SEQ ID NO: 5977) and KDR mRNA site 726 (SEQ ID NO: 5978) were tested in the corneal model along with their attenuated controls. Five treatment groups were assigned to examine the effects of five doses of each test substance over a dose range of 1-100 µg on VEGF-stimulated angiogenesis. Negative (30 µM VEGF soaked filter disk and intraconjunctival injection of 600 nL sterile water) and no stimulus (Tris-soaked filter disk and intraconjunctival injection of sterile water) control groups were also included. Each group consisted of five animals (10 eyes) receiving the same treatment.

Quantitation of angiogenic response. Five days after disk implantation, animals were euthanized following im administration of 0.4 mg/kg atropine and corneas were digitally imaged. The neovascular surface area (NSA, expressed in pixels) was measured postmortem from blood-filled corneal vessels using computerized morphometry (Image Pro Plus, Media Cybernetics, v2.0). The individual mean NSA was determined in triplicate from three regions of identical size in the area of maximal neovascularization between the filter disk and the limbus. The number of pixels corresponding to the blood-filled corneal vessels in these regions was summated to produce an index of NSA. A group mean NSA was then calculated. Data from each treatment group were normalized to VEGF/ribozyme vehicle-treated control NSA and finally expressed as percent inhibition of VEGF-induced angiogenesis.

Statistics. After determining the normality of treatment group means, group mean percent inhibition of VEGF-induced angiogenesis was subjected to a one-way analysis of variance. This was followed by two post-hoc tests for significance including Dunnett's (comparison to VEGF control) and Tukey-Kramer (all other group mean comparisons) at alpha = 0.05. Statistical analyses were performed using JMP v.3.1.6 (SAS Institute).

RESULTS

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Ribozyme-mediated reduction of VEGF-induced cell proliferation: Ribozyme cleavage of Flt-1 or KDR mRNA should result in a decrease in the density of cell surface VEGF receptors. This decrease should limit VEGF binding and consequently interfere with the mitogenic signaling induced by VEGF. To determine if cell proliferation was impacted by anti-Flt-1 and/or anti-KDR ribozyme treatment, proliferation assays using cultured human microvascular cells were carried out. Ribozymes included in the proliferation assays were

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initially chosen by their ability to decrease the level of VEGF binding to treated cells. In these initial studies, ribozymes targeting 20 sites in the coding region of each mRNA were screened. The most effective ribozymes against two sites in each target, Flt-1 sites 1358 and 4229 and KDR sites 726 and 3950, were included in the proliferation assays reported here. In addition, attenuated analogs of each ribozyme were used as controls. These attenuated controls are still capable of binding to the mRNA target since the binding arm sequence is maintained. However, these controls have two nucleotide changes in the core sequence that substantially reduce their ability to carry out the cleavage reaction.

The active ribozymes tested decreased the relative proliferation of HMVEC-d after VEGF stimulation, an effect that increased with ribozyme concentration. This concentration dependency was not observed following treatment with the attenuated controls designed for these sites. In fact, little or no change in cell growth was noted following treatment with the attenuated controls, even though these controls can still bind to the specific target sequences. At 200 nM, there was a distinct "window" between the anti-proliferative effects of each ribozyme and its attenuated control; a trend also observed at lower doses. This window of inhibition of proliferation (56-77% based on total cells/well) reflects the contribution of ribozyme-mediated activity. In comparison, no effect of anti-Flt-1 or anti-KDR ribozymes was noted on bFGF-stimulated cell proliferation. Moreover, an irrelevant, but active, ribozyme whose binding sequence is not found in either Flt-1 or KDR mRNA had no effect in this assay. These data are consistent with the basic ribozyme mechanism in which binding and cleavage are necessary components. Although the relative surface distribution of Flt-1 and KDR receptors in this cell type is not known, the antiproliferative effects of these ribozymes indicate that, at least in cell culture, both receptors are functionally coupled to proliferation.

Specific reduction of Flt-1 or KDR mRNA by ribozyme treatment: To confirm that anti-Flt-1 and anti-KDR ribozymes reduce their respective mRNA targets, cellular levels of Flt-1 or KDR were quantified using an RNAse protection assay with specific Flt-1 or KDR probes. For each target, one ribozyme/attenuated control pair was chosen for continued study. Exposure of HMVEC-d to active ribozyme targeting Flt-1 site 4229 decreased Flt-1 mRNA, but not KDR mRNA. Likewise, treatment with the active ribozyme targeting KDR site 726 decreased KDR, but not Flt-1 mRNA. Both ribozymes decreased the level of their respective target RNA by greater than 50%. The degree of reduction associated with the corresponding attenuated controls was not greater than 13%.

In vitro activity of anti-Flt and anti-KDR ribozymes.

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To confirm further the necessity of an active ribozyme core, in vitro cleavage activities were determined for the Flt-1 site 4229 ribozyme and the KDR site 726 ribozyme as well as their paired attenuated controls. The first order rate constants calculated from the time-course of short substrate cleavage for the anti-Flt-1 ribozyme and its attenuated control were $0.081 \pm 0.0007 \text{ min}^{-1}$ and $0.001 \pm 6 \times 10^{-5} \text{ min}^{-1}$, respectively. For the anti-KDR ribozyme and its paired control, the first order rate constants were $0.434 \pm 0.024 \text{ min}^{-1}$ and $0.002 \pm 1 \times 10^{-4} \text{ min}^{-1}$, respectively. Although the attenuated controls retain a very slight level of cleavage activity under these optimized conditions, the decrease in in vitro cleavage activity between each active ribozyme and its paired attenuated control is about two orders of magnitude. Thus, an active core is essential for cleavage activity in vitro and is also necessary for ribozyme activity in cell culture.

Ribozyme-mediated reduction of VEGF-induced angiogenesis in vivo. To assess whether ribozymes targeting VEGF receptor mRNA could impact the complex process of angiogenesis, prototypic anti-Flt-1 and KDR ribozymes that were identified in cell culture studies were screened in a rat corneal pocket assay of VEGF-induced angiogenesis. In this assay, comeas implanted with VEGF-containing filter disks exhibited a robust neovascular response in the corneal region between the disk and the corneal limbus (from which the new vessels emerge). Disks containing a vehicle solution elicited no angiogenic response. In separate studies, intraconjunctival injections of sterile water vehicle did not affect the magnitude of the VEGF-induced angiogenic response. In addition, ribozyme injections alone did not induce angiogenesis.

The dose-related effects of anti-Flt-1 or KDR ribozymes on the VEGF-induced angiogenic response were then examined. The antiangiogenic effect of the anti-Flt-1 (site 4229) and KDR (site 726) ribozymes and their attenuated controls over a dose range from 1 to 100 µg, respectively was determined. For both ribozymes, the maximal antiangiogenic response (48 and 36% for anti-Flt-1 and KDR ribozymes, respectively) was observed at a dose of 10 µg.

The anti-Flt-1 ribozyme produced a significantly greater antiangiogenic response than its attenuated control at 3 and 10 μ g (p<0.05). Its attenuated control exhibited a small but significant antiangiogenic response at doses above 10 μ g compared to vehicle treated VEGF controls (p<0.05). At its maximum, this response was not significantly greater than that observed with the lowest dose of active anti-Flt-1 ribozyme. The anti-KDR ribozyme significantly inhibited angiogenesis from 3 to 30 μ g (p<0.05). The anti-KDR attenuated control had no significant effect at any dose tested.

Example 3. In vivo inhibition of tumor growth and metastases by VEGF-R ribozymes.

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Lewis Lung Carcinoma Mouse Model: Ribozymes were chemically synthesized as described above. The sequence of ANGIOZYME™ bound to its target RNA is shown in Figure 1.

The tumors in this study were derived from a cell line (LLC-HM) which gives rise to reproducible numbers of spontaneous lung metastases when propagated in vivo. The LLC-HM line was obtained from Dr. Michael O'Reilly, Harvard University. Tumor neovascularization in Lewis lung carcinoma has been shown to be VEGF-dependent. Tumors from mice bearing LLC-HM (selected for the highly metastatic phenotype by serial propagation) were harvested 20 days post-inoculation. A tumor brei suspension was prepared from these tumors according to standard protocols. On day 0 of the study, 0.5 x 10⁶ viable LLC-HM tumor cells were injected subcutaneously (sc) into the dorsum or flank of previously untreated mice (100 µL injectate). Tumors were allowed to grow for a period of 3 days prior to initiating continuous intravenous administration of saline or 30 mg/kg/d ANGIOZYMETM via Alzet mini-pumps. One set of animals was dosed from days 3 to 17, inclusive. Tumor length and width measurements and volumes were calculated according to the formula: Volume = 0.5(length)(width)². At post-inoculation day 25, animals were euthanized and lungs harvested. The number of lung macrometastatic nodules was counted. It should be noted that metastatic foci were quantified 8 days after the cessation of dosing. Ribozyme solutions were prepared to deliver to another set of animals 100, 10, 3, or 1 mg/kg/day of ANGIOZYME™ via Alzet mini-pumps. A total of 10 animals per dose or saline control group were surgically implanted on the left flank with osmotic mini-pumps prefilled with the respective test solution three days following tumor inoculation. Pumps were attached to indwelling jugular vein catheters.

Figure 2 shows the antitumor effects of ANGIOZYMETM. There is a statistically significant inhibition (p < 0.05) of primary LLC-HM tumor growth in tumors grown in the flank regions compared to saline control. ANGIOZYME™ significantly reduced (p < 0.05) the number of lung metastatic foci in animals inoculated either in the flank regions. Figure 3 illustrates the dose-dependent anti-metastatic effect of ANGIOZYME™ compared to saline control.

B. Mouse Colorectal Cancer Model. KM12L4a-16 is a human colorectal cancer cell line. On day 0 of the study, 0.5 x 10⁶ KM12L4a-16 cells were implanted into the spleen of nude mice. Three days after tumor inoculation, Alzet minipumps were implanted and continuous subcutaneous delivery of either saline or 12, 36 or 100 mg/kg/ day of

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ANGIOZYMETM was initiated. On day 5, the spleens containing the primary tumors were removed. On day 18, the Alzet minipumps were replaced with fresh pumps so that delivery of saline or ANGIOZYMETM was continuous over a 28 day period from day 3 to day 32. Animals were euthanized on day 41 and the liver tumor burden was evaluated.

Following treatment with 100 mg/kg/day of ANGIOZYMETM, there was a significant reduction in the incidence and median number of liver metastasis (Figure 4). In saline-treated animals, the median number of metastases was 101. However, at the high dose of ANGIOZYMETM (100 mg/kg/day), the median number of metastases was zero.

Example 4: Effect of ANGIOZYMETM alone or in combination with chemotherapeutic agents in the mouse Lewis Lung Carcinoma Model.

Methods

Tumor inoculations. Male C57/BL6 mice, age 6 to 8 weeks, were inoculated subcutaneously in the flank with 5×10^5 LLC-HM cells from brei preparations made from tumors grown in mice.

Ribozymes and controls. RPI.4610, also known as ANGIOZYMETM (SEQ ID NO: 5977), is an anti-Flt-1 ribozyme that targets site 4229 in the human Flt-1 receptor mRNA (EMBL accession no. X51602). The controls tested include RPI.13141, an attenuated version of RPI.4610 in which four nucleotides in the catalytic core are changed so that the cleavage activity is dramatically decreased. RPI.13141, however, maintains the base composition and binding arms of RPI.4610 and so is still capable of binding to the target site. The second control (RPI.13030) also has changes to the catalytic core (three) to inhibit cleavage activity, but in addition the sequence of the binding arms has been scrambled so that it can no longer bind to the target sequence. One nucleotide in the arm of RPI.13030 is also changed to maintain the same base composition as RPI.4610.

Ribozyme administrations. Ribozymes and controls were resuspended in normal saline. Administration was initiated seven days following tumor inoculation. Animals either received a daily subcutaneous injection (30 mg/kg test substance) from day 7 to day 20 or were instrumented with an Alzet osmotic minipump (12 µL/day flow rate) containing a solution of ribozyme or control. Subcutaneous infusion pumps delivered the test substances (30 mg/kg/day) from day 7 to 20 (14-day pumps, 420 mg/kg total test substance) or days 7-34 (28-day pumps, 840 mg/kg total test substance). Where indicated, chemotherapeutic agents were given in combination with ribozyme treatment. Cyclophosphamide was given by intraperitoneal administration on days 7, 9 and 11 (125 mg/kg). Gemcitabine was given by

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intraperitoneal administration on days 8, 11 and 14 (125 mg/kg). Untreated, uninstrumented animals were used as comparison. Five animals were included in each group.

Results

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The antiangiogenic ribozyme, ANGIOZYME™, was tested in a model of Lewis lung carcinoma alone and in combination with two chemotherapeutic agents. Previously (see above), 30 mg/kg/day ANGIOZYME™ alone was determined to inhibit both primary tumor growth and lung metastases in a highly metastatic variant of Lewis lung (continuous 14-day iv deliveryvia Alzet minipump, manuscript in preparation).

In this study, 30 mg/kg/day ANGIOZYMETM delivered either as a daily subcutaneous bolus injection or as a continuous infusion from an Alzet minipump resulted in a delay in tumor growth. On average, tumor growth to 500 mm³ was delayed by ~7 days in animals being treated with ANGIOZYMETM compared to an untreated group. Growth of tumors in animals being treated with either of two attenuated controls was delayed by only ~ 2 days.

ANGIOZYMETM delivered by subcutaneous bolus was also tested in combination with either Gemcytabine or cyclophosphamide. Tumor growth delay increased by about 3 days in the presence of combination therapy with ANGIOZYMETM and Gemcytabine over the effects of either treatment alone. The combination of ANGIOZYMETM and cyclophosphamide did not increase tumor growth delay over that of cyclophosphamide alone, however, suboptimal doses of cyclophosphamide were not included in this study. Neither of the attenuated controls increased the effect of the chemotherapeutic agents.

The effect of ANGIOZYMETM on metastases to the lung was also determined in the presence and absence of additional chemotherapeutic treatment. Macrometastases to the lungs were counted in two animals in each treatment group on day 20. In the presence of ANGIOZYMETM, with or without a chemotherapeutic agent, the lung metastases were reduced to zero. Treatment with either Gemcytabine or cyclophosphamide alone (mean number of metastases 4.5 and 4, respectively) were not as effective as ANGIOZYMETM alone or when used in combination with ANGIOZYMETM. Neither of the attenuated controls increased the effect of the chemotherapeutic agents.

The effect on metastases to the lung was also determined following continuous treatment with ANGIOZYME™. At day 20, an average of ~8 macrometastases were noted in the treatment groups which had been instrumented with Alzet minipumps (either 14- or 28-day pumps). This is a decrease in metastases of ~50% from the untreated group. Since

ANGIOZYMETM delivered by a daily subcutaneous bolus resulted in zero metastases (Fig.4) in the two animals counted, it is possible that the additional burden of being instrumented with the minipump contributes to a slightly decreased response to ANGIOZYMETM.

Example 5: Identification of Potential Target Sites in Human VEGFR1 and/or VEGFR2 RNA

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The sequence of human VEGFR1 and/or VEGFR2 genes are screened for accessible sites using a computer-folding algorithm. Regions of the RNA that do not form secondary folding structures and contain potential enzymatic nucleic acid molecule and/or antisense binding/cleavage sites are identified. An exemplary sequence of an enzymatic nucleic acid molecule of the invention is shown in Formula I and/or Formula II (SEQ ID Nos: 5977 and 5978, respectively). Other nucleic acid molecules and targets contemplated by the invention are described in Pavco et al., US Patent Application No. 09/870,161, incorporated by reference herein in its entirety. Similarly, other nucleic acid molecules of the invention, including antisense, aptamers, dsRNA, siRNA, and/or 2,5-A chimeras, can be designed to modulate the expression of the nucleic acid targets described in Pavco et al., US Patent Application No. 09/870,161.

Example 6: Selection of Enzymatic Nucleic Acid Cleavage Sites in Human VEGFR1 and/or VEGFR2 RNA

Enzymatic nucleic acid molecule target sites are chosen by analyzing sequences of human VEGFR1 receptor (for example Genbank Accession No. NM_002019), and VEGFR2 receptor (for example Genbank Accession No. NM_002253) genes and prioritizing the sites on the basis of folding. Enzymatic nucleic acid molecules are designed that can bind each target and are individually analyzed by computer folding (Christoffersen et al., 1994 J. Mol. Struc. Theochem, 311, 273; Jaeger et al., 1989, Proc. Natl. Acad. Sci. USA, 86, 7706) to assess whether the enzymatic nucleic acid molecule sequences fold into the appropriate secondary structure. Those enzymatic nucleic acid molecules with unfavorable intramolecular interactions between the binding arms and the catalytic core can be eliminated from consideration. As discussed herein, varying binding arm lengths can be chosen to optimize activity. Generally, at least 4 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Example 7: Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of VEGFR1 and/or VEGFR2 RNA

Enzymatic nucleic acid molecules and antisense constructs are designed to anneal to various sites in the RNA message. The binding arms of the enzymatic nucleic acid molecules are complementary to the target site sequences described above, while the antisense constructs are fully complementary to the target site sequences described above. RNAi molecules (dsRNA) likewise have one strand of RNA or a portion of RNA complementarity to the target site sequence or a portion of the target site sequence. For example, complementarity within the double-strand RNAi structure is formed from two separate individual RNA strands or from self-complementary areas of a topologically closed, individual RNA strand which can be optionally circular. The nucleic acid molecules were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman et al., (1987 J. Am. Chem. Soc., 109, 7845), Scaringe et al., (1990 Nucleic Acids Res., 18, 5433) and Wincott et al., supra, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were typically >98%.

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Nucleic acid molecules are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, Methods Enzymol. 180, 51). Nucleic acid molecules of the invention are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott et al., supra; the totality of which is hereby incorporated herein by reference) and are resuspended in water. Examples of sequences of chemically synthesized enzymatic nucleic acid molecules are shown in Formula I (SEQ ID NO: 5977), Formula II (SEQ ID NO: 5978) and in Pavco et al., US Patent Application No. 09/870,161.

Example 8: Enzymatic Nucleic Acid Molecule Cleavage of VEGFR1 and/or VEGFR2 RNA Target in vitro

Enzymatic nucleic acid molecules targeted to the human VEGFR1 and/or VEGFR2 RNA are designed and synthesized as described above. These enzymatic nucleic acid molecules can be tested for cleavage activity in vitro, for example, using the following procedure. The target sequences and the nucleotide location within the VEGFR1 and/or VEGFR2 RNA are described in Pavco et al., US Patent Application No. 09/870,161.

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for enzymatic nucleic acid molecule cleavage assay is prepared by in vitro transcription in the presence of [a-32p] CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'-32P-end

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labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified enzymatic nucleic acid molecule in enzymatic nucleic acid molecule cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction was initiated by adding the 2X enzymatic nucleic acid molecule mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM enzymatic nucleic acid molecule, i.e., enzymatic nucleic acid molecule excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by enzymatic nucleic acid molecule cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

15 Example 9: Phase I/II Study of Repetitive Dosing of ANGIOZYME™ Targeting the VEGFR1 (FLT-1) Receptor of VEGF

A ribozyme therapeutic agent ANGIOZYME™ (SEQ ID NO: 5977), was assessed by daily subcutaneous administration in a phase I/II trial for 31 patients with refractory solid tumors. Demographic information relating to patients enrolled in the study are shown in Table III. The primary study endpoint was to determine the safety and maximum tolerated dose of ANGIOZYME™. Secondary endpoints assessed ANGIOZYME™ pharmacokinetics and clinical response. Patients were treated at the following doses: 3 patients received doses of 10 mg/m²/day, 4 patients received 30 mg/m²/day, 20 patients received 100 mg/m²/day, and 4 patients received 300 mg/m²/day. All but one patient were dosed for a minimum of 29 consecutive days with 24-hour pharmacokinetic analyses on Day 1 and 29. Clinical response The data from 20 patients indicated was assessed monthly. Results ANGIOZYME™ was well tolerated, with no systemic adverse events. Figure 5 shows the plasma concentration profile of ANGIOZYME™ after a single subcutaneous dose of 10, 30, 100, or 300 mg/m². The pharmacokinetic parameters of ANGIOZYME™ after subcutaneous bolus administration are outlined in Table IV. An MTD (maximum tolerated dose) could not be established. One patient in the 300 mg/m²/d group experienced a grade 3 injection site reaction. Patients in the other groups experienced intermittent grade 1 and grade 2 injection site reactions with erythema and induration. No systemic or laboratory toxicities were observed. Pharmacokinetic analyses demonstrated dose-dependent plasma concentrations with good bioavailability (70-90%), t1/2 = 209-384 min, and no accumulation after repeated doses. To date, 17/28 (61%) of evaluable patients have had stable disease for periods of one to six months and two patients (nasopharyngeal squamous cell carcinoma and melanoma) had minor clinical responses. The patient with nasopharyngeal carcinoma demonstrated central tumor necrosis as indicated by MRI. The longest period of treatment thus far has been 8 months for two patients at 100 mg/m²/d (breast, peritoneal mesothelioma).

Example 10: Down-regulation of VEGFR1 gene expression to treat gynecologic neovascularization dependent conditions

One patient in the Phase I/II trial described in Example 19 was menstruating prior to enrollment in the ANGIOZYME™ monotherapy trial. After 1-2 months on trial, the patient's menstrual cycles ceased. The patient remained on trial for approximately 11 months and did not menstruate. The patient then went off the trial for about 4 months and the menstrual Re-enrollment in the ANGIOZYMETM trial resulted in the patient's cycles resumed. menstrual cycle stopping again. This clinical observation suggests that ANGIOZYMETM is interfering with the patient's menstrual cycle, perhaps by inhibiting neovascularization of uterine tissue. This data also suggests that ANGIOZYMETM has a direct effect on the endometrial tissue or an effect on LH/FSH stimulation. These results suggest the treatment or control, using ANGIOZYME™ (SEQ ID NO: 5977) and/or other nucleic acid molecules of the instant invention, of various clinical targets and/or processes associated with female reproduction and gynecologic neovascularization, such as endometriosis, birth control, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), menopausal dysfunction, endometrial carcinoma or other condition associated with the expression of VEGFR1 and/or VEGFR2 VEGF receptors.

Example 11: Down-regulation of VEGFR1 in clinical setting

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Twenty-seven of the patients enrolled in the Phase I/II trial described in Example 19 had day 1 (baseline) and day 43 (six-week) serum samples assayed for VEGFR1 biomarker. VEGFR1 levels were statistically different after six weeks of ANGIOZYME treatment (Figure 9). Although statistical testing involving all 27 patients showed statistical support for effects, not all patients presented with elevated levels of VEGF-R1. Since the effects of ANGIOZYME on VEGF-R1 may only be demonstrated when sufficient levels are present at baseline, a cutoff of 100 pg/mL was chosen and changes in this VEGF-R1 were re-analyzed. Ten of the 27 patients presented with baseline VEGF-R1 levels in excess of 100 pg/mL. For this subgroup VEGF-R1 levels were lower by 3-fold, p<001. After six weeks of treatment the average (geometric mean) of VEGF-R1 decreased for this subgroup from 419 pg/ml to

132pg/ml, p<.001. These results show that treatment with ANGIOZYME results in a statistically significant reduction in VEGFR1 expression.

Example 22: In vivo inhibition of neovascularization in an ocular animal model by VEGF-R ribozymes.

Summary of the Mouse Model: A mouse model of proliferative retinopathy (Aiello et 5 al., 1995, Proc. Natl. Acad. Sci. USA 92: 10457-10461; Robinson et al., 1996, Proc. Natl. Acad. Sci. USA 93: 4851-4856; Pierce et al., 1996, Archives of Ophthalmology 114: 1219-1228) in which neovascularization of the mouse retina is induced by exposure of 7-day old mice to 75% oxygen followed by a return to normal room air. The initial period in high oxygen causes an obliteration of developing blood vessels in the retina. Exposure to room air 10 five days later is perceived as hypoxia by the now underperfused retina. The result is an immediate upregulation of VEGF mRNA and VEGF protein (between 6-12 hours) followed by an extensive retinal neovascularization that peaks in ~5 days. Although this model is more representative of retinopathy of prematurity than diabetic retinopathy, it is an accepted small animal model in which to study neovascular pathophysiology of the retina. In fact, 15 intravitreal injection of certain antisense DNA constructs targeting VEGF mRNA have been found to be antiangiogenic in this model, as were soluble VEGF receptor chimeric proteins designed to bind VEGF in the vitreous humor (Aiello et al., 1995, Proc. Natl. Acad. Sci. USA 92: 10457-10461; Robinson et al., 1996, Proc. Natl. Acad. Sci. USA 93: 4851-4856; Pierce et al., 1996, Archives of Ophthalmology 114: 1219-1228). 20

Summary of experiment: The effect of an anti-KDR/Flk-1 ribozyme on the peak level of neovascularization was tested in the mouse model described above. As shown in Figure 10, P7 mice were removed from the hyperoxic chamber and the mice received two intraocular injections (P12 and P13) in the right eye of 10 µg RPI.4731, the anti-KDR/Flk-1 ribozyme. The left eye of each mouse was treated as a control and received intraocular injections of saline. Five days after being exposed to room air, neovascular nuclei in the retina of both eyes were counted. Data are presented in Figure 11. There was a significant decrease in retinal neovascularization (~40%) compared to the control, saline-injected eyes.

RPL4731 sequence and chemical composition: 5'-u_sa_sc_s a_sau ucU GAu Gag gcg aaa gcc Gaa Aag aca aB-3' (SEQ ID NO: 5978)

where:

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uppercase G, A = ribonucleotides lowercase = 2'-OMe U = 2'-C-allyl uridine WO 02/096927 PCT/US02/17674

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B = inverted abasic nucleotide

S = phosphorothioate internucleotide linkage

Indications

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- 1) Tumor angiogenesis: Angiogenesis has been shown to be necessary for tumors to grow into pathological size (Folkman, 1971, PNAS 76, 5217-5221; Wellstein & Czubayko, 1996, Breast Cancer Res and Treatment 38, 109-119). In addition, it allows tumor cells to travel through the circulatory system during metastasis. Increased levels of gene expression of a number of angiogenic factors such as vascular endothelial growth factor (VEGF) have been reported in vascularized and edema-associated brain tumors (Berkman et al., 1993 J. Clini. Invest. 91, 153). A more direct demostration of the role of VEGF in tumor angiogenesis was demonstrated by Jim Kim et al., 1993 Nature 362,841 wherein, monoclonal antibodies against VEGF were successfully used to inhibit the growth of rhabdomyosarcoma, glioblastoma multiforme cells in nude mice. Similarly, expression of a dominant negative mutated form of the flt-1 VEGF receptor inhibits vascularization induced by human glioblastoma cells in nude mice (Millauer et al., 1994, Nature 367, 576). Specific tumor/cancer types that can be targeted using the nucleic acid molecules of the invention include but are not limited to the tumor/cancer types described under Diagnosis in Table III.
- 2) Ocular diseases: Neovascularization has been shown to cause or exacerbate ocular diseases including but not limited to, macular degeneration, neovascular glaucoma, diabetic retinopathy, myopic degeneration, and trachoma (Norrby, 1997, APMIS 105, 417-437). Aiello et al., 1994 New Engl. J. Med. 331, 1480, showed that the ocular fluid, of a majority of patients suffering from diabetic retinopathy and other retinal disorders, contains a high concentration of VEGF. Miller et al., 1994 Am. J. Pathol. 145, 574, reported elevated levels of VEGF mRNA in patients suffering from retinal ischemia. These observations support a direct role for VEGF in ocular diseases. Other factors including those that stimulate VEGF synthesis may also contribute to these indications.
 - 3) <u>Dermatological Disorders</u>: Many indications have been identified which may by angiogenesis dependent including but not limited to psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, pot-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, and Osler-Weber-Rendu syndrome (Norrby, *supra*). Intradermal injection of the angiogenic factor b-FGF demonstrated angiogenesis in nude mice (Weckbecker et al., 1992, *Angiogenesis: Key principles-Science-Technology-Medicine*, ed R. Steiner) Detmar et al., 1994 J. Exp. Med. 180, 1141 reported that VEGF and its receptors were over-expressed in

psoriatic skin and psoriatic dermal microvessels, suggesting that VEGF plays a significant role in psoriasis.

4) Rheumatoid arthritis: Immunohistochemistry and in situ hybridization studies on tissues from the joints of patients suffering from rheumatoid arthritis show an increased level of VEGF and its receptors (Fava et al., 1994 J. Exp. Med. 180, 341). Additionally, Koch et al., 1994 J. Immunol. 152, 4149, found that VEGF-specific antibodies were able to significantly reduce the mitogenic activity of synovial tissues from patients suffering from rheumatoid arthritis. These observations support a direct role for VEGF in rheumatoid arthritis. Other angiogenic factors including those of the present invention may also be involved in arthritis.

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5) Endometriosis: Various studies indicate that VEGF is directly implicated in endometriosis. In one study, VEGF concentrations measured by ELISA in peritoneal fluid were found to be significantly higher in women with endometriosis than in women without endometriosis (24.1 ± 15 ng/ml vs 13.3 ± 7.2 ng/ml in normals). In patients with endometriosis, higher concentrations of VEGF were detected in the proliferative phase of the menstrual cycle (33 ± 13 ng/ml) compared to the secretory phase (10.7 ± 5 ng/ml). The cyclic variation was not noted in fluid from normal patients (McLaren et al., 1996, Human Reprod. 11, 220-223). In another study, women with moderate to severe endometriosis had significantly higher concentrations of peritoneal fluid VEGF than women without endometriosis. There was a positive correlation between the severity of endometriosis and the concentration of VEGF in peritoneal fluid. In human endometrial biopsies, VEGF expression increased relative to the early proliferative phase approximately 1.6-, 2-, and 3.6-fold in midproliferative, late proliferative, and secretory endometrium (Shifren et al., 1996, J. Clin. Endocrinol. Metab. 81, 3112-3118).

In a third study, VEGF-positive staining of human ectopic endometrium was shown to be localized to macrophages (double immunofluorescent staining with CD14 marker). Peritoneal fluid macrophages demonstrated VEGF staining in women with and without endometriosis. However, increased activation of macrophages (acid phosphatatse activity) was demonstrated in fluid from women with endometriosis compared with controls. Peritoneal fluid macrophage conditioned media from patients with endometriosis resulted in significantly increased cell proliferation ([³H] thymidine incorporation) in HUVEC cells compared to controls. The percentage of peritoneal fluid macrophages with VEGFR2 mRNA was higher during the secretory phase, and significantly higher in fluid from women with endometriosis (80 ± 15%) compared with controls (32 ± 20%). Flt-mRNA was detected in

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peritoneal fluid macrophages from women with and without endometriosis, but there was no difference between the groups or any evidence of cyclic dependence (McLaren et al., 1996, J. Clin. Invest. 98, 482-489).

In the early proliferative phase of the menstrual cycle, VEGF has been found to be expressed in secretory columnar epithelium (estrogen-responsive) lining both the oviducts and the uterus in female mice. During the secretory phase, VEGF expression was shown to have shifted to the underlying stroma composing the functional endometrium. In addition to examining the endometrium, neovascularization of ovarian follicles and the corpus luteum, as well as angiogenesis in embryonic implantation sites have been analyzed. For these processes, VEGF was expressed in spatial and temporal proximity to forming vasculature (Shweiki et al., 1993, J. Clin. Invest. 91, 2235-2243).

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The present body of knowledge in VEGFR1 and/or VEGFR2 research indicates the need for methods to assay VEGFR1 and/or VEGFR2 activity and for compounds that can regulate VEGFR1 and/or VEGFR2 expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related of VEGF, VEGFR1 and/or VEGFR2 levels. In addition, the nucleic acid molecules can be used to treat disease state related to VEGF and/or VEGFR, such as VEGFR1 and/or VEGFR2 levels.

Particular processes, diseases, or conditions that can be associated with VEGFR1 and/or VEGFR2 levels include, but are not limited to, gynecologic neovascularization, such as endometriosis, endometrial carcinoma, gynecologic bleeding disorders, irregular menstrual cycles, ovulation, premenstrual syndrome (PMS), menopausal dysfunction, other diseases and conditions discussed herein, and other diseases or conditions that are related to or respond to the levels of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2, in a cell or tissue, alone or in combination with other therapies

The use of GnRH (gonadotropin releasing hormone) agonists, Lupron Depot (Leuprolide Acetate), Synarel (naferalin acetate), Zolodex (goserelin acetate), Suprefact (buserelin acetate), Danazol, or oral contraceptives including, but not limited to, Depo-Provera or Provera (medroxyprogesterone acetate), or any other estrogen/progesterone contraceptive, are all non-limiting examples of compounds and methods that can be combined with or used in conjunction with the nucleic acid molecules of the instant invention. Various chemotherapies can be readily combined with nucleic acid molecules of the invention for the treatment of endometrial carcinoma. Common chemotherapies that can be combined with nucleic acid molecules of the instant invention include various combinations of cytotoxic drugs to kill the

cancer cells. These drugs include but are not limited to paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubin, fluorouracil carboplatin, edatrexate, gemcitabine, vinorelbine etc. Those skilled in the art will recognize that other drug compounds and therapies can be readily combined with the nucleic acid molecules of the instant invention and are hence within the scope of the instant invention.

Animal Models

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There are several animal models in which the anti-angiogenesis effect of nucleic acids of the present invention, such as ribozymes, directed against VEGF-R mRNAs can be tested. Typically, a corneal model has been used to study angiogenesis in rat and rabbit since recruitment of vessels can easily be followed in this normally avascular tissue (Pandey et al., 1995 Science 268: 567-569). In these models, a small Teflon or Hydron disk pretreated with an angiogenesis factor (e.g. bFGF or VEGF) is inserted into a pocket surgically created in the cornea. Angiogenesis is monitored 3 to 5 days later. Ribozymes directed against VEGF-R mRNAs would be delivered in the disk as well, or dropwise to the eye over the time course of the experiment. In another eye model, hypoxia has been shown to cause both increased expression of VEGF and neovascularization in the retina (Pierce et al., 1995 Proc. Natl. Acad. Sci. USA. 92: 905-909; Shweiki et al., 1992 J. Clin. Invest. 91: 2235-2243).

In human glioblastomas, it has been shown that VEGF is at least partially responsible for tumor angiogenesis (Plate et al., 1992 Nature 359, 845). Animal models have been developed in which glioblastoma cells are implanted subcutaneously into nude mice and the progress of tumor growth and angiogenesism is studied (Kim et al., 1993 supra; Millauer et al., 1994 supra).

Another animal model that addresses neovascularization involves Matrigel, an extract of basement membrane that becomes a solid gel when injected subcutaneously (Passaniti et al., 1992 Lab. Invest. 67: 519-528). When the Matrigel is supplemented with angiogenesis factors such as VEGF, vessels grow into the Matrigel over a period of 3 to 5 days and angiogenesis can be assessed. Ribozymes directed against VEGF-R mRNAs can be delivered in the Matrigel to assess anti-angiogesis effect.

Several animal models exist for screening of anti-angiogenic agents. These include corneal vessel formation following comeal injury (Burger et al., 1985 Cornea 4: 35-41; Lepri, et al., 1994 J. Ocular Pharmacol. 10: 273-280; Ormerod et al., 1990 Am. J. Pathol. 137: 1243-1252) or intracorneal growth factor implant (Grant et al., 1993 Diabetologia 36: 282-291; Pandey et al. 1995 supra; Zieche et al., 1992 Lab. Invest. 67: 711-715), vessel

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growth into Matrigel matrix containing growth factors (Passaniti et al., 1992 supra), female reproductive organ neovascularization following hormonal manipulation (Shweiki et al., 1993 Clin. Invest. 91: 2235-2243), several models involving inhibition of tumor growth in highly vascularized solid tumors (O'Reilly et al., 1994 Cell 79: 315-328; Senger et al., 1993 Cancer and Metas. Rev. 12: 303-324; Takahasi et al., 1994 Cancer Res. 54: 4233-4237; Kim et al., 1993 supra), and transient hypoxia-induced neovascularization in the mouse retina (Pierce et al., 1995 Proc. Natl. Acad. Sci. USA. 92: 905-909).

The comea model, described in Pandey et al. *supra*, is the most common and well characterized anti-angiogenic agent efficacy screening model. This model involves an avascular tissue into which vessels are recruited by a stimulating agent (growth factor, thermal or alkalai burn, endotoxin). The corneal model utilizes the intrastromal corneal implantation of a Teflon pellet soaked in a VEGF-Hydron solution to recruit blood vessels toward the pellet which can be quantitated using standard microscopic and image analysis techniques. To evaluate their anti-angiogenic efficacy, ribozymes are applied topically to the eye or bound within Hydron on the Teflon pellet itself. This avascular cornea as well as the Matrigel (see below) provide for low background assays. While the corneal model has been performed extensively in the rabbit, studies in the rat have also been conducted.

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The mouse model (Passaniti et al., supra) is a non-tissue model which utilizes Matrigel, an extract of basement membrane (Kleinman et al., 1986) or Millipore[®] filter disk, which can be impregnated with growth factors and anti-angiogenic agents in a liquid form prior to injection. Upon subcutaneous administration at body temperature, the Matrigel or Millipore[®] filter disk forms a solid implant. VEGF embedded in the Matrigel or Millipore[®] filter disk would be used to recruit vessels within the matrix of the Matrigel or Millipore[®] filter disk which can be processed histologically for endothelial cell specific vWF (factor VIII antigen) immunohistochemistry, Trichrome-Masson stain, or hemoglobin content. Like the cornea, the Matrigel or Millipore[®] filter disk are avascular; however, it is not tissue. In the Matrigel or Millipore[®] filter disk model, ribozymes are administered within the matrix of the Matrigel or Millipore[®] filter disk to test their anti-angiogenic efficacy. Thus, delivery issues in this model, as with delivery of ribozymes by Hydron- coated Teflon pellets in the rat cornea model, are minimized due to the homogeneous presence of the ribozyme within the respective matrix.

These models offer a distinct advantage over several other angiogenic models listed previously. The ability to use VEGF as a pro-angiogenic stimulus in both models is highly desirable since ribozymes target only VEGFr mRNA. In other words, the involvement of

other non-specific types of stimuli in the cornea and Matrigel models is not advantageous from the standpoint of understanding the pharmacologic mechanism by which the anti-VEGFr mRNA ribozymes produce their effects. In addition, the models allow for testing the specificity of the anti-VEGFr mRNA ribozymes by using either aFGF or bFGF as a pro-angiogenic factor. Vessel recruitment using FGF should not be affected in either model by anti-VEGFr mRNA ribozymes. Other models of angiogenesis, including vessel formation in the female reproductive system using hormonal manipulation (Shweiki et al., 1993 supra); a variety of vascular solid tumor models which involve indirect correlations with angiogenesis (O'Reilly et al., 1994 supra; Senger et al., 1993 supra; Takahasi et al., 1994 supra; Kim et al., 1993 supra); and retinal neovascularization following transient hypoxia (Pierce et al., 1995 supra), were not selected for efficacy screening due to their non-specific nature, although they can be useful models due to a demonstrated correlation between VEGF and angiogenesis.

Other model systems to study tumor angiogenesis is reviewed by Folkman, 1985 Adv. Cancer. Res.. 43, 175.

Use of murine models

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For a typical systemic study involving 10 mice (20 g each) per dose group, 5 doses (1, 3, 10, 30 and 100 mg/kg daily over 14 days continuous administration), approximately 400 mg of ribozyme, formulated in saline would be used. A similar study in young adult rats (200 g) would require over 4 g. Parallel pharmacokinetic studies involve the use of similar quantities of ribozymes further justifying the use of murine models.

Ribozymes and Lewis lung carcinoma and B-16 melanoma murine models

Identifying a common animal model for systemic efficacy testing of ribozymes is an efficient way of screening ribozymes for systemic efficacy.

The Lewis lung carcinoma and B-16 murine melanoma models are well accepted models of primary and metastatic cancer and are used for initial screening of anti-cancer agents. These murine models are not dependent upon the use of immunodeficient mice, are relatively inexpensive, and minimize housing concerns. Both the Lewis lung and B-16 melanoma models involve subcutaneous implantation of approximately 10⁶ tumor cells from metastatically aggressive tumor cell lines (Lewis lung lines 3LL or D122, LLc-LN7; B-16-BL6 melanoma) in C57BL/6J mice. Alternatively, the Lewis lung model can be produced by the surgical implantation of tumor spheres (approximately 0.8 mm in diameter). Metastasis

also can be modeled by injecting the tumor cells directly intraveneously. In the Lewis lung model, microscopic metastases can be observed approximately 14 days following implantation with quantifiable macroscopic metastatic tumors developing within 21-25 days. The B-16 melanoma exhibits a similar time course with tumor neovascularization beginning 4 days following implantation. Since both primary and metastatic tumors exist in these models after 21-25 days in the same animal, multiple measurements can be taken as indices of efficacy. Primary tumor volume and growth latency as well as the number of micro- and macroscopic metastatic lung foci or number of animals exhibiting metastases can be quantitated. The percent increase in lifespan can also be measured. Thus, these models provide suitable primary efficacy assays for screening systemically administered ribozymes/ribozyme formulations.

In the Lewis lung and B-16 melanoma models, systemic pharmacotherapy with a wide variety of agents usually begins 1-7 days following tumor implantation/inoculation with either continuous or multiple administration regimens. Concurrent pharmacokinetic studies can be performed to determine whether sufficient tissue levels of ribozymes can be achieved for pharmacodynamic effect to be expected. Furthermore, primary tumors and secondary lung metastases can be removed and subjected to a variety of *in vitro* studies (*i.e.* target RNA reduction).

Flt-1, KDR and/or flk-1 protein levels can be measured clinically or experimentally by FACS analysis. Flt-1, KDR and/or flk-1 encoded mRNA levels can be assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block flt-1, KDR and/or flk-1 protein encoding mRNAs and therefore result in decreased levels of flt-1, KDR and/or flk-1 activity by more than 20% in vitro can be identified.

Ribozymes and/or genes encoding them are delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments (see above).

Subjects can be treated by locally administering nucleic acids targeted against VEGF-R by direct injection. Routes of administration include, but are not limited to, intravascular, intramuscular, subcutaneous, intraarticular, aerosol inhalation, oral (tablet, capsule or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery.

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Surgically induced models of endometriosis have been developed in rats, mice, and rabbits. Non-human primates demonstrate spontaneous endometriosis, but surgical induction can also be used. In addition to the surgical technique, cycle monitoring can be performed by daily vaginal cytology in primates. For all of the surgically induced models of endometriosis, the following general procedure is used. An initial laparotomy is performed to implant tissue from a donor animal. A portion of one uterine horn (or one complete horn in the case of mice) is removed. The endometrium of this piece of uterus is separated from the myometrium and cut into small segments (4-10 mm2). Segments (approximately 3) are sutured to various locations within the abdominal cavity (peritoneum, intestinal mesentery vessels, uterus, broad ligament). Cummings and Metcalf (1996) attached whole segments of mouse uterus without separating the endometrium from the myometrium. Implants are allowed to grow for 3-6 A second laparotomy is sometimes performed to verify development of weeks. endometriosis-like foci (vascularization and cysts filled with clear fluid). This second laparotomy was done in the studies by Quereda et al., (1996) and Stoeckemann et al., (1995). After 3-6 weeks post-surgery and/or following visualization of endometriosis, drug treatment is initiated and continued for a prescribed period of time. At the termination of these studies, animals are euthanized. Endpoints include, but are not limited to, changes in the surface area of the implants and tissue mass of the ectopic endometrial implants (see for example Brogniez et al., 1995, Human Reprod. 10, 927-931; Cummings et al., 1996, Tox. Appl. Pharm. 138, 131-139; Cummings and Metcalf, 1996, Proc. Soc. Exp. Biol. Med. 212, 332-337; D'Hooghe et al., 1996, Fertility and Sterility. 66, 809-813; Quereda et al., 1996, Eur. J. Obstet. Gynecol. Rep. Biol. 67, 35-40; and Stoeckemann et al., 1995, Human Reprod. 10, 3264-3271).

Combination therapies

Gemcytabine and cyclophosphamide are non-limiting examples of chemotherapeutic agents that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other anti-angiogenic and/or anti-cancer compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. ribozymes and antisense molecules) and are hence within the scope of the instant invention. Such compounds and therapies are well known in the art (see for example Cancer: Principles

and Pranctice of Oncology, Volumes 1 and 2, eds Devita, V.T., Hellman, S., and Rosenberg, S.A., J.B. Lippincott Company, Philadelphia, USA; incorporated herein by reference) and include, without limitations, folates, antifolates, pyrimidine analogs, fluoropyrimidines, purine analogs, adenosine analogs, topoisomerase I inhibitors, anthrapyrazoles, retinoids, antibiotics, anthacyclins, platinum analogs, alkylating agents, nitrosoureas, plant derived compounds such as vinca alkaloids, epipodophyllotoxins, tyrosine kinase inhibitors, taxols, radiation therapy, surgery, nutritional supplements, gene therapy, radiotherapy, for example 3D-CRT, immunotoxin therapy, for example ricin, and monoclonal antibodies. Specific examples of chemotherapeutic compounds than can be combined with or used in conjuction with the nucleic acid molecules of the invention include but are not limited to Paclitaxel; Docetaxel; Methotrexate; Doxorubin; Edatrexate; Vinorelbine; Tomaxifen; Leucovorin; 5fluoro uridine (5-FU); Irinotecan (CAMPTOSAR® or CPT-11 or Camptothecin-11 or Campto); Cisplatin; Carboplatin; Amsacrine; Cytarabine; Bleomycin; Mitomycin C; Dactinomycin; Mithramycin; Hexamethylmelamine; Dacarbazine; L-asperginase; Nitrogen mustard; Melphalan, Chlorambucil; Busulfan; Ifosfamide; 4-hydroperoxycyclophosphamide, Thiotepa; Tamoxifen, Herceptin; IMC C225; ABX-EGF: and combinations thereof.

Diagnostic uses

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The nucleic acid molecules of this invention (e.g., enzymatic nucleic acid molecules) can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of VEGF and/or VEGFr, such as VEGFR1 and/or VEGFR2 RNA in a cell. The close relationship between enzymatic nucleic acid molecule activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple enzymatic nucleic acid molecules described in this invention, one can map nucleotide changes which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with enzymatic nucleic acid molecules can be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments can lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple enzymatic nucleic acid molecules targeted to different genes, enzymatic nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of enzymatic nucleic acid molecules and/or other chemical or biological molecules). Other in

vitro uses of enzymatic nucleic acid molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with VEGF, VEGFR1 and/or VEGFR2-related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with an enzymatic nucleic acid molecule using standard methodology.

In a specific example, enzymatic nucleic acid molecules which cleave only wild-type or mutant forms of the target RNA are used for the assay. The first enzymatic nucleic acid molecule is used to identify wild-type RNA present in the sample and the second enzymatic nucleic acid molecule is used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both enzymatic nucleic acid molecules to demonstrate the relative enzymatic nucleic acid molecule efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis requires two enzymatic nucleic acid molecules, two substrates and one unknown sample which is combined into six reactions. The presence of cleavage products is determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., VEGFR1 and/or VEGFR2) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively. The use of enzymatic nucleic acid molecules in diagnostic applications contemplated by the instant invention is described, for example, in Usman et al., US Patent Application No. 09/877,526, George et al., US Patent Nos. 5,834,186 and 5,741,679, Shih et al., US Patent No. 5,589,332, Nathan et al., US Patent No 5,871,914, Nathan and Ellington, International PCT publication No. WO 00/24931, Breaker et al., International PCT Publication Nos. WO 00/26226 and 98/27104, and Sullenger et al., US Patent Application Serial No. 09/205,520.

Additional Uses

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Uses of sequence-specific enzymatic nucleic acid molecules of the instant invention can have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans et al., 1975 Ann. Rev. Biochem. 44:273). For example,

the pattern of restriction fragments can be used to establish sequence relationships between two related RNAs, and large RNAs can be specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the enzymatic nucleic acid molecule is ideal for cleavage of RNAs of unknown sequence. Applicant has described the use of nucleic acid molecules to down-regulate gene expression of target genes in bacterial, microbial, fungal, viral, and eukaryotic systems including plant, or mammalian cells.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

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One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

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In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

5 Other embodiments are within the following claims.

TABLE !

Characteristics of Ribozymes

Group I Introns

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage

site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena* thermophila rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number of nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1 and 2)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table II:

	A. 2.5 µmol Syn	thesis Cycle	A. 2.5 µmol Synthesis Cycle ABI 394 Instrument	.	
Reagent	Equivalents	Amount	Wait Time* DNA Wait Time* 2'- O-methyl	Wait Time* 2'- O-methyl	Wait Time* RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
lodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucade	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA VA	NA V	A A

75 July 15 Co. 17			Wait Time* DNA Wait Time* 2'- O-methyl	Wait Time* 2'- O-methyl	Wait Time*
31 μL 45 sec 31 μL 45 sec 5 124 μL 5 sec 124 μL 5 sec 732 μL 10 sec 6 244 μL 15 sec	<u>0</u>	31 PL 31 PL 124 PL 732 PL 244 PL	45 sec 45 sec 5 sec 10 sec	233 sec 233 min 5 sec 5 sec 10 sec 15 sec	465 sec 465 sec 5 sec 10 sec 15 sec

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

C. 0 Equivalents	Amount 2.64 mL NA 2.64 mL NA Amount Amount	NA NA Wait Time* DNA	NA NA NA NA Mait Time* 2'-O-methyl	NA .O. Walt Time* Ribo
DNA/2'-O-metnyl/Klbo 22/33/66	40/60/120 ul	eo sec	180 sec	360sec
70/105/210	40/60/120 µL	oes 09	180 min	360 sec
265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
	80/120/120	100 sec	200 sec	200 sec
	1150/1150/1150 µL	NA V	NA V	A V

* Wait time does not include contact time during delivery.

Table III: Patient Demographics

Dose cohort	Pt#	Δα2 .	Sex	Diagnosis	Doses
(mg/m²) 10	1001	Age 49	F	NSC Lung	29
10	1002	65	F	liposarcoma	120
10	1002	49	M	nasopharyngeal CA	109
30	1004	35	M	non-small cell lung	1
30	1005	45	F	melanoma (ocular)	113
30	1006	57	M	colon	199
30	1007	39	F	epitheliod hemangioendothelioma	198
100	1008	52	M	adrenal CA	57
100	1009	44	F	breast	35
100	1010	62	F	renal	134
300	1011	24	F	melanoma	31
300	1012	57	M	renal cell	178
300	1013	53	M	nasopharyngeal SCCA	29
300	1014	64	F	peritoneal mesothelioma	324
100	1015	65	M	melanoma	140
100	1016	77	F	breast	265
100	1017		F	melanoma	35
100	1018	26	F	melanoma	7
100	1019	69	F_	endometrial sarcoma	500
100	1020	65	M	carcinoid	124
100	1021	59	M	gallbladder adeno carcinoma	34
100	1022	43	M	colorectal	8
100	1023	78	F	breast	50
100	1024	40	F	parotid adenocarcinoma	285
100	1025	52	F	breast	71
100	1026	39	F	breast	34
100	1027	55	F	breast	36
100	1028	52	M	melanoma	29
100	1029	38	M	pancreatic	36
100	1030	83	M	melanoma	41
100	1031	50	M	medullary thyroid	108

One patient taken off study due to progressive disease. Allowed to resume ANGIOZYME on a compassionate basis.

As of September 1, 2001, all patients were off study. (Although one patient resumed treatment per above note)

Table V: Human FLT DNAzyme and Substrate Sequence

Pos	Substrate	Seq ID No	DNAzyme	Seq IID No
17	UCCUCUCG G CUCCUCCC	1	GGGAGGAG GGCTAGCTACAACGA CGAGAGGA	1703
28	CCUCCCCG G CAGCGGCG	2	CGCCGCTG GGCTAGCTACAACGA CGGGGAGG	
31	CCCCGGCA G CGGCGGCG	3	CGCCGCCG GGCTAGCTACAACGA TGCCGGGG	
34	CGGCAGCG G CGGCGGCU	4	AGCCGCCG GGCTAGCTACAACGA CGCTGCCG	1706
37	CAGCGGCG G CGGCUCGG	5	CCGAGCCG GGCTAGCTACAACGA CGCCGCTG	
40	CGGCGGCG G CUCGGAGC	6	GCTCCGAG GGCTAGCTACAACGA CGCCGCCG	
47	GGCUCGGA G CGGGCUCC	7	GGAGCCCG GGCTAGCTACAACGA TCCGAGCC	
51	CGGAGCGG G CUCCGGGG	В	CCCCGGAG GGCTAGCTACAACGA CCGCTCCG	
59	GCUCCGGG G CUCGGGUG	9	CACCCGAG GGCTAGCTACAACGA CCCGGAGC	
65	GGGCUCGG G UGCAGCGG	10	CCGCTGCA GGCTAGCTACAACGA CCGAGCCC	
67	GCUCGGGU G CAGCGGCC	11	GGCCGCTG GGCTAGCTACAACGA ACCCGAGC	
	CGGGUGCA G CGGCCAGC	12	GCTGGCCG GGCTAGCTACAACGA TGCACCCC	
70	GUGCAGCG G CCAGCGGG	1.3	CCCGCTGG GGCTAGCTACAACGA CGCTGCAC	
73	AGCGGCCA G CGGGCCUG	14	CAGGCCCG GGCTAGCTACAACGA TGGCCGC	
77	GCCAGCGG G CCUGGCGG	15	CCGCCAGG GGCTAGCTACAACGA CCGCTGGC	
81	CGGGCCUG G CGGCGAGG	16	CCTCGCCG GGCTAGCTACAACGA CAGGCCC	
86	GCCUGGCG G CGAGGAUU	17	AATCCTCG GGCTAGCTACAACGA CGCCAGG	
89	CGGCGAGG A UUACCCGG	18	CCGGGTAA GGCTAGCTACAACGA CCTCGCC	
95	CGAGGAUU A CCCGGGGA	19	TCCCCGGG GGCTAGCTACAACGA AATCCTC	
98	CCGGGGAA G UGGUUGUC	20	GACAACCA GGCTAGCTACAACGA TTCCCCG	
108	GGGAAGUG G UUGUCUCC	21	GGAGACAA GGCTAGCTACAACGA CACTTCC	
111	AAGUGGUU G UCUCCUGG	22	CCAGGAGA GGCTAGCTACAACGA AACCACT	
114	GUCUCCUG G CUGGAGCC	23	GGCTCCAG GGCTAGCTACAACGA CAGGAGA	
122	UGGCUGGA G CCGCGAGA	24	TCTCGCGG GGCTAGCTACAACGA TCCAGCC	
128	CUGGAGCC G CGAGACGG	25	CCGTCTCG GGCTAGCTACAACGA GGCTCCA	
131	GCCGCGAG A CGGGCGCU	26	AGCGCCCG GGCTAGCTACAACGA CTCGCGG	
136	CGAGACGG G CGCUCAGG	27	CCTGAGCG GGCTAGCTACAACGA CCGTCTC	
140	AGACGGGC G CUCAGGGC	28	GCCCTGAG GGCTAGCTACAACGA GCCCGTC	
142		29	GCCCCGCG GGCTAGCTACAACGA CCTGAGC	
149	CGCUCAGG G CGCGGGGC	30	CGGCCCCG GGCTAGCTACAACGA GCCCTG	
151	CUCAGGGC G CGGGGCCG	31	GCCGCCGG GGCTAGCTACAACGA CCCGCGC	
156	GGCGCGGG G CCGGCGGC	32	CGCCGCCG GGCTAGCTACAACGA CGGCCCC	
160	CGGGGCCG G CGGCGCA		GTTCGCCG GGCTAGCTACAACGA CGCCGGC	
163	GGCCGGCG G CGGCGAAC	33	CTCGTTCG GGCTAGCTACAACGA CGCCGC	
166	CGGCGGCG G CGAACGAG	34	TCCTCTCG GGCTAGCTACAACGA TCGCCG	
170	GGCGGCGA A CGGAGUGU	35	AGAGTCCG GGCTAGCTACAACGA CCTCTC	
178	ACGAGAGG A CUCUGGGG	37	CGCCAGAG GGCTAGCTACAACGA CCGTCC	
182	GAGGACGG A CUCUGGCG		CCCGGCCG GGCTAGCTACAACGA CAGAGT	
188	GGACUCUG G CGGCCGGG	38	CGACCGG GGCTAGCTACAACGA CGCCAG	
191	CUCUGGCG G CCGGGUCG	39	GCCAACGA GGCTAGCTACAACGA CCGGCC	
196	GCGGCCGG G UCGUUGGC	40	CCGGCCAA GGCTAGCTACAACGA GACCCG	
199	GCCGGGUC G UUGGCCGG		TCCCCGG GGCTAGCTACAACGA CAACGA	
203	GGUCGUUG G CCGGGGGA		TGCCCGG GGCTAGCTACAACGA CAACGA	
212	CCGGGGGA G CGCGGGCA		GGTGCCG GGCTAGCTACAACGA TCCCCC	
214	GGGGGAGC G CGGGCACC		GCCCGGTG GGCTAGCTACAACGA GCTCCC	
218	GAGCGCGG G CACCGGGC			
220	GCGCGGC A CCGGGCGA		TCGCCCGG GGCTAGCTACAACGA GCCCGC	
225	GGCACCGG G CGAGCAGG		CCTGCTCG GGCTAGCTACAACGA CCGGTG	
229	CCGGGCGA G CAGGCCGC	48	GCGGCCTG GGCTAGCTACAACGA TCGCCC	GG 175

Table IV Pharmacokinetic parameters of ANGIOZYME after bolus subcutaneous administration.

	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2/11/2	30 m	Jan 2	100 m	2/m²	300 mg/m ²	g/m²
		C.S	Moon	CD.	Mean	as	Mean	SD
	Mean	O.D.	Mean	3	The state of the s		100	2 02
	0.43	0.07	0.62	0.28	3.17	0.69	8.91	7.73
Day-Line Charax (ug/mL)	6.5		,	i i	74.44	300	80.87	21.68
ATIC+ (ma*hr/m)	2.60	1.43	6.04	2.70	34.I4	7.70	10.70	
Company of the second of the s		70.0	7 00	1 66	37.51	1.91	101.57	13.47
AUCinf (ug*hr/mL)	4.40	00.0	(2)	20.1			700	7 20
() () () () () () () () () ()	3 63	0.70	7.32	6.94	4.58	0.02	9.70	0.20
t(1/2) (nr)	20.0		272	0 0	2.96	0.61	2.99	0.43
CL/F (L/hr/m²)	7.74	0.00	5.75	7:72			500	671
E 27 . X 4	0.25	0 10	1.17	0.53	3.23	0.35	8.93	0./1
Day 29 CHRX (ugmr)	0.0	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \		,	71 07	101	110 42	65.84
ATIC+ (ma*hr/m)	2.11	1.31	7.29	1.16	31.8/	1.71	77.77	
Company Sp. 1004		1 2 1	8 54	2 46	33.61	2.16	132.73	78./9
AUCinf (ug*hr/mL)	3.38	1.31	10.0	2	,,,,	36.0	707	0.70
1(4 M) (A-1)	4 40	1,60	3.26	1.01	4.66	0.33	+7.1	
(III) (7/T)				700	2 2 1	0.56	2.72	1.40
CL/F (L/hr/m²)	2.49	1.48	5.09	0.74	3.41			

			CGACGCGG GGCTAGCTACAACGA CTGCTCGC 1751
233	GCGAGCAG G CCGCGUCG	49	GCGCGACG GGCTAGCTACAACGA GGCCTGCT 1752
236	AGCAGGCC G CGUCGCGC	50	GCGCG100 GGG111011121112111
238	CAGGCCGC G UCGCGCUC	51	GAGCGCAT COCTACTOR
241	GCCGCGUC G CGCUCACC	52	GG1GAGCO COCALACTACA CASCA CASCA
243	CGCGUCGC G CUCACCAU	53	Alddidno cocinediation
247	UCGCGCUC A CCAUGGUC	54	GACCATGG GGCTAGCTACAACGA GAGCGCGA 1756
250	CGCUCACC A UGGUCAGC	55	GCTGACCA GGCTAGCTACAACGA GGTGAGCG 1757
253	UCACCAUG G UCAGCUAC	56	GTAGCTGA GGCTAGCTACAACGA CATGGTGA 1758
257	CAUGGUCA G CUACUGGG	57	CCCAGTAG GGCTAGCTACAACGA TGACCATG 1759
260	GGUCAGCU A CUGGGACA	58	TGTCCCAG GGCTAGCTACAACGA AGCTGACC 1760
266	CUACUGGG A CACCGGGG	59	CCCCGGTG GGCTAGCTACAACGA CCCAGTAG 1761
268	ACUGGGAC A CCGGGGUC	60	GACCCCGG GGCTAGCTACAACGA GTCCCAGT 1762
274	ACACCGGG G UCCUGCUG	61	CAGCAGGA GGCTAGCTACAACGA CCCGGTGT 1763
279	GGGGUCCU G CUGUGCGC	62	GCGCACAG GGCTAGCTACAACGA AGGACCCC 1764
282	GUCCUGCU G UGCGCGCU	63	AGCGCGCA GGCTAGCTACAACGA AGCAGGAC 1765
284	CCUGCUGU G CGCGCUGC	64	GCAGCGCG GGCTAGCTACAACGA ACAGCAGG 1766
286	UGCUGUGC G CGCUGCUC	65	GAGCAGCG GGCTAGCTACAACGA GCACAGCA 1767
288	CUGUGCGC G CUGCUCAG	66	CTGAGCAG GGCTAGCTACAACGA GCGCACAG 1768
291	UGCGCGCU G CUCAGCUG	67	CAGCTGAG GGCTAGCTACAACGA AGCGCGCA 1769
296	GCUGCUCA G CUGUCUGC	68	GCAGACAG GGCTAGCTACAACGA TGAGCAGC 1770
299	GCUCAGCU G UCUGCUUC	69	GAAGCAGA GGCTAGCTACAACGA AGCTGAGC 1771
303	AGCUGUCU G CUUCUCAC	70	GTGAGAAG GGCTAGCTACAACGA AGACAGCT 1772
310	UGCUUCUC A CAGGAUCU	71	AGATCCTG GGCTAGCTACAACGA GAGAAGCA 1773
315	CUCACAGG A UCUAGUUC	72	GAACTAGA GGCTAGCTACAACGA CCTGTGAG 1774
	AGGAUCUA G UUCAGGUU	73	AACCTGAA GGCTAGCTACAACGA TAGATCCT 1775
320	UAGUUCAG G UUCAAAAU	74	ATTTTGAA GGCTAGCTACAACGA CTGAACTA 1776
326	GGUUCAAA A UUAAAAGA	75	TCTTTTAA GGCTAGCTACAACGA TTTGAACC 1777
333	AUUAAAAG A UCCUGAAC	76	GTTCAGGA GGCTAGCTACAACGA CTTTTAAT 1778
341	GAUCCUGA A CUGAGUUU	77	AAACTCAG GGCTAGCTACAACGA TCAGGATC 1779
348	UGAACUGA G UUUAAAAG	78	CTTTTAAA GGCTAGCTACAACGA TCAGTTCA 1780
353	UUUAAAAG G CACCCAGC	79	GCTGGGTG GGCTAGCTACAACGA CTTTTAAA 1781
362		80	GTGCTGGG GGCTAGCTACAACGA GCCTTTTA 1782
364	UAAAAGGC A CCCAGCAC	81	ATGATGTG GGCTAGCTACAACGA TGGGTGCC 1783
369	GGCACCCA G CACAUCAU	82	GCATGATG GGCTAGCTACAACGA GCTGGGTG 1784
371	CACCCAGC A CAUCAUGC	<u> </u>	TTGCATGA GGCTAGCTACAACGA GTGCTGGG 1785
373	CCCAGCAC A UCAUGCAA	83	TGCTTGCA GGCTAGCTACAACGA GATGTGCT 1786
376	AGCACAUC A UGCAAGCA	84	IGCIIGCA CCCIACCIIICALICAT CONTROL 1705
378	CACAUCAU G CAAGCAGG	85	CCIGCIIG GOCIACCIIICIII
382	UCAUGCAA G CAGGCCAG	86	CTGGCCTG GGCTAGCTACAACGA TTGCATGA 1788 GTGTCTGG GGCTAGCTACAACGA CTGCTTGC 1789
386	GCAAGCAG G CCAGACAC	87	GIGICIGG GGCIAGCIIGIIGI
391	CAGGCCAG A CACUGCAU	88	MIGCHGIG GGCIMGCIMGIAGA: GAGGA
393	GGCCAGAC A CUGCAUCU	89	AGAIGCAG COCIAGOILLOIL
396	CAGACACU G CAUCUCCA	90	IGGROATO COCITICOTTATION
398	GACACUGC A UCUCCAAU	91	ATTGGAGA GGCTAGCTACAACGA GCAGTGTC 1793
405	CAUCUCCA A UGCAGGGG	92	CCCCTGCA GGCTAGCTACAACGA TGGAGATG 1794
407	UCUCCAAU G CAGGGGG	93	CCCCCTG GGCTAGCTACAACGA ATTGGAGA 1795
418	GGGGGGAA G CAGCCCAU	94	ATGGGCTG GGCTAGCTACAACGA TTCCCCCC 1796
421	GGGAAGCA G CCCAUAAA	95	TTTATGGG GGCTAGCTACAACGA TGCTTCCC 1797
425	AGCAGCCC A UAAAUGGU	96	ACCATTTA GGCTAGCTACAACGA GGGCTGCT 1798
429	GCCCAUAA A UGGUCUUU	97	AAAGACCA GGCTAGCTACAACGA TTATGGGC 1799
432	CAUAAAUG G UCUUUGCC	98	GGCAAAGA GGCTAGCTACAACGA CATTTATG 1800
	UGGUCUUU G CCUGAAAU	99	ATTTCAGG GGCTAGCTACAACGA AAAGACCA 1801
438	1 page construction		ACTCACCA GGCTAGCTACAACGA TTCAGGCA 1802

448	CUGAAAUG G UGAGUAAG	101	CTTACTCA GGCTAGCTACAACGA CATTTCAG 18	303
452	AAUGGUGA G UAAGGAAA	102	TTTCCTTA GGCTAGCTACAACGA TCACCATT 1	B04
461	UAAGGAAA G CGAAAGGC	103	GCCTTTCG GGCTAGCTACAACGA TTTCCTTA 1	805
468	AGCGAAAG G CUGAGCAU	104	ATGCTCAG GGCTAGCTACAACGA CTTTCGCT 1	806
473	AAGGCUGA G CAUAACUA	105	TAGTTATG GGCTAGCTACAACGA TCAGCCTT 1	807
475	GGCUGAGC A UAACUAAA	106	TTTAGTTA GGCTAGCTACAACGA GCTCAGCC 1	808
478	UGAGCAUA A CUAAAUCU	107	AGATTTAG GGCTAGCTACAACGA TATGCTCA 1	809
483	AUAACUAA A UCUGCCUG	108	CAGGCAGA GGCTAGCTACAACGA TTAGTTAT 1	810
487	CUAAAUCU G CCUGUGGA	109	TCCACAGG GGCTAGCTACAACGA AGATTTAG 1	811
491	AUCUGCCU G UGGAAGAA	110	TTCTTCCA GGCTAGCTACAACGA AGGCAGAT 1	.812
500	UGGAAGAA A UGGCAAAC	111	GTTTGCCA GGCTAGCTACAACGA TTCTTCCA 1	.813
503	AAGAAAUG G CAAACAAU	112	ATTGTTTG GGCTAGCTACAACGA CATTTCTT 1	814
507	AAUGGCAA A CAAUUCUG	113	CAGAATTG GGCTAGCTACAACGA TTGCCATT	1815
510	GGCAAACA A UUCUGCAG	114	CTGCAGAA GGCTAGCTACAACGA TGTTTGCC	1816
515	ACAAUUCU G CAGUACUU	115	AAGTACTG GGCTAGCTACAACGA AGAATTGT	1817
518	AUUCUGCA G UACUUUAA	116	TTAAAGTA GGCTAGCTACAACGA TGCAGAAT	1818
520	UCUGCAGU A CUUUAACC	117	GGTTAAAG GGCTAGCTACAACGA ACTGCAGA	1819
526	GUACUUUA A CCUUGAAC	118		1820
533	AACCUUGA A CACAGCUC	119	the state of the s	1821
535	CCUUGAAC A CAGCUCAA	120		1822
538	UGAACACA G CUCAAGCA	121		1823
544	CAGCUCAA G CAAACCAC	122		1824
548	UCAAGCAA A CCACACUG	123		1825
	AGCAAACC A CACUGGCU	124		1826
551	CAAACCAC A CUGGCUUC	125		1827
553	CCACACUG G CUUCUACA	126		1828
557	UGGCUUCU A CAGCUGCA	127		1829
563	CUUCUACA G CUGCAAAU	128	ATTTGCAG GGCTAGCTACAACGA TGTAGAAG	1830
566	CUACAGCU G CAAAUAUC	129	GATATTTG GGCTAGCTACAACGA AGCTGTAG	1831
569	AGCUGCAA A UAUCUAGC	130	GCTAGATA GGCTAGCTACAACGA TTGCAGCT	1832
573	CUGCAAAU A UCUAGCUG	131	CAGCTAGA GGCTAGCTACAACGA ATTTGCAG	1833
575	AAUAUCUA G CUGUACCU	132	AGGTACAG GGCTAGCTACAACGA TAGATATT	1834
580	AUCUAGCU G UACCUACU	133	AGTAGGTA GGCTAGCTACAACGA AGCTAGAT	1835
583		134	GAAGTAGG GGCTAGCTACAACGA ACAGCTAG	1836
585	CUAGCUGU A CCUACUUC	135	CTTTGAAG GGCTAGCTACAACGA AGGTACAG	1837
589	CUGUACCU A CUUCAAAG	136	AGATTCTG GGCTAGCTACAACGA TTCCTTCT	1838
607	AGAAGGAA A CAGAAUCU	137	ATTGCAGA GGCTAGCTACAACGA TCTGTTTC	1839
612	GAAACAGA A UCUGCAAU	138	ATAGATTG GGCTAGCTACAACGA AGATTCTG	1840
616	CAGAAUCU G CAAUCUAU		TATATAGA GGCTAGCTACAACGA TGCAGATT	1841
619	AAUCUGCA A UCUAUAUA	139	TATATAGA GGCTAGCTACAACGA IGCAGATT TAAATATA GGCTAGCTACAACGA AGATTGCA	1842
623	UGCAAUCU A UAUAUUUA	140		1843
625	CAAUCUAU A UAUUUAUU	141	CTATAAA GGCTAGCTACAACGA ATAGATTG	1844
627	AUCUAUAU A UUUAUUAG	142	ATCACTAA GGCTAGCTACAACGA AAATATAT	
631	AUAUAUUU A UUAGUGAU	143	CTGTATCA GGCTAGCTACAACGA TAATAAAT	
635	AUUUAUUA G UGAUACAG	144	TACCTGTA GGCTAGCTACAACGA CACTAATA	
638	UAUUAGUG A UACAGGUA	145	TACCIGIA GGCTAGCTACAACGA CACTAATA TCTACCTG GGCTAGCTACAACGA ATCACTAA	
640	UUAGUGAU A CAGGUAGA	146	AAGGTCTA GGCTAGCTACAACGA ATCACTAA	ļ
644	UGAUACAG G UAGACCUU	147	ACGAAAGG GGCTAGCTACAACGA CTACCTGT	
648	ACAGGUAG A CCUUUCGU	148		
655	GACCUUUC G UAGAGAUG	149	CATCTCTA GGCTAGCTACAACGA GAAAGGTC	
661	UCGUAGAG A UGUACAGU	150	ACTGTACA GGCTAGCTACAACGA CTCTACGA	
663	GUAGAGAU G UACAGUGA	151	TCACTGTA GGCTAGCTACAACGA ATCTCTAC	
665	AGAGAUGU A CAGUGAAA	152	TTTCACTG GGCTAGCTACAACGA ACATCTCT	1034

			GATTTCA GGCTAGCTACAACGA TGTACATC 1855	l
668	GAUGUACA G UGAAAUCC		TCGGGGA GGCTAGCTACAACGA TTCACTGT 1856	
673	ACAGUGAA A UCCCCGAA		TICGGGGA CCCTIACTIACTIACTIA	
682	UCCCCGAA A UUAUACAC		GIGIAIAA COCAMOCINGAIGE. ECC.	1
685	CCGAAAUU A UACACAUG		MIGIGIA COCIACCIACIANTICALIA	1
687	GAAAUUAU A CACAUGAC		GICAIGIG GCCIIIGCIIICAI	1
689	AAUUAUAC A CAUGACUG		CHG1CH10 GOCINGCINGING	1
691	UUAUACAC A UGACUGAA	159	TICAGICA GOCIAGCIAGRICAL COCA	┥
694	UACACAUG A CUGAAGGA		ICCIICAG GOCIAGOTAGATAGAT	┥
708	GGAAGGGA G CUCGUCAU	161	Although Cocinocination: 2001200	1
712	GGGAGCUC G UCAUUCCO	162	GOGANICA COCINGCING	4
715	AGCUCGUC A UUCCCUGC	163	GCAGGGAA GGCIAGCIAGTIGGI GIAGGG	-
722	CAUUCCCU G CCGGGUUA	164	TANCCCGO GOCTAGCTAGGT.	4
727	CCUGCCGG G UUACGUCA	165	IGACGIAA GGCIAGCIAGIIIGGI GIGG	-
730	GCCGGGUU A CGUCACCU	166	AGGIGACG CGCIAGCIAGAIAGAI	-
732	CGGGUUAC G UCACCUAA	167	TTAGGTGA GGCTAGCTACAACGA GTAACCCG 1869	-
735	GUUACGUC A CCUAACAU	168	ATGTTAGG GGCTAGCTACAACGA GACGTAAC 1870	-
740	GUCACCUA A CAUCACUG	169	CAGTGATG GGCTAGCTACAACGA TAGGTGAC 1871	-
742	CACCUAAC A UCACUGUU	170	AACAGTGA GGCTAGCTACAACGA GTTAGGTG 1872	-
745	CUAACAUC A CUGUUACU	171	AGTAACAG GGCTAGCTACAACGA GATGTTAG 1873	_
748	ACAUCACU G UUACUUUA	172	TAAAGTAA GGCTAGCTACAACGA AGTGATGT 1874	_
751	UCACUGUU A CUUUAAAA	173	TTTTAAAG GGCTAGCTACAACGA AACAGTGA 1875	
762	UUAAAAAA G UUUCCACU	174	AGTGGAAA GGCTAGCTACAACGA TTTTTTAA 1876	_
768	AAGUUUCC A CUUGACAC	175	GTGTCAAG GGCTAGCTACAACGA GGAAACTT 1877	
773	UCCACUUG A CACUUUGA	176	TCAAAGTG GGCTAGCTACAACGA CAAGTGGA 1878	_
775	CACUUGAC A CUUUGAUC	177	GATCAAAG GGCTAGCTACAACGA GTCAAGTG 1879	_
781	ACACUUUG A UCCCUGAU	178	ATCAGGGA GGCTAGCTACAACGA CAAAGTGT 1880	
788	GAUCCCUG A UGGAAAAC	179	GTTTTCCA GGCTAGCTACAACGA CAGGGATC 1881	_
795	GAUGGAAA A CGCAUAAU	180	ATTATGCG GGCTAGCTACAACGA TTTCCATC 1882	_
797	UGGAAAAC G CAUAAUCU	181	AGATTATG GGCTAGCTACAACGA GTTTTCCA 1883	_
799	GAAAACGC A UAAUCUGG	182	CCAGATTA GGCTAGCTACAACGA GCGTTTTC 1884	_
802	AACGCAUA A UCUGGGAC	183	GTCCCAGA GGCTAGCTACAACGA TATGCGTT 1885	
809	AAUCUGGG A CAGUAGAA	184	TTCTACTG GGCTAGCTACAACGA CCCAGATT 1886	\dashv
812	CUGGGACA G UAGAAAGG	185	CCTTTCTA GGCTAGCTACAACGA TGTCCCAG 1887	
821	UAGAAAGG G CUUCAUCA	186	TGATGAAG GGCTAGCTACAACGA CCTTTCTA 1888	
826	AGGGCUUC A UCAUAUCA	187	TGATATGA GGCTAGCTACAACGA GAAGCCCT 1889	
829	GCUUCAUC A UAUCAAAU	188	ATTTGATA GGCTAGCTACAACGA GATGAAGC 1890	
831	UUCAUCAU A UCAAAUGC	189	GCATTTGA GGCTAGCTACAACGA ATGATGAA 1891	
836	CAUAUCAA A UGCAACGU	190	ACGTTGCA GGCTAGCTACAACGA TTGATATG 1892	
838	UAUCAAAU G CAACGUAC	191	GTACGTTG GGCTAGCTACAACGA ATTTGATA 1893	
841	CAAAUGCA A CGUACAAA	192	TTTGTACG GGCTAGCTACAACGA TGCATTTG 1894	
843	AAUGCAAC G UACAAAGA	193	TCTTTGTA GGCTAGCTACAACGA GTTGCATT 1895	5
845	UGCAACGU A CAAAGAAA	194	TTTCTTTG GGCTAGCTACAACGA ACGTTGCA 1896	>
853	ACAAAGAA A UAGGGCUU		AAGCCCTA GGCTAGCTACAACGA TTCTTTGT 1897	7
	GAAAUAGG G CUUCUGAC	196	GTCAGAAG GGCTAGCTACAACGA CCTATTTC 1898	
858		197	TTCACAGG GGCTAGCTACAACGA CAGAAGCC 189	9
865		198	TTGCTTCA GGCTAGCTACAACGA AGGTCAGA 1900	0
869		199	GACTGTTG GGCTAGCTACAACGA TTCACAGG 190:	1
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877			CCCATTGA GGCTAGCTACAACGA TGTTGCTT 190	
880	TO THE PERSON OF		AATGCCCA GGCTAGCTACAACGA TGACTGTT 190	
884			TACAAATG GGCTAGCTACAACGA CCATTGAC 190	
888			TATACAAA GGCTAGCTACAACGA GCCCATTG 190	
890	CAAUGGGC A UUUGUAUA	204	21111201111	

894	GGGCAUUU G UAUAAGAC	205	GTCTTATA GGCTAGCTACAACGA AAATGCCC 19	07
896	GCAUUUGU A UAAGACAA	206	TTGTCTTA GGCTAGCTACAACGA ACAAATGC 19	80
901	UGUAUAAG A CAAACUAU	207	ATAGTTTG GGCTAGCTACAACGA CTTATACA 19	09
905	UAAGACAA A CUAUCUCA	208	TGAGATAG GGCTAGCTACAACGA TTGTCTTA 19	10
908	GACAAACU A UCUCACAC	209	GTGTGAGA GGCTAGCTACAACGA AGTTTGTC 19	11
913	ACUAUCUC A CACAUCGA	210	TCGATGTG GGCTAGCTACAACGA GAGATAGT 19	12
915	UAUCUCAC A CAUCGACA	211	TGTCGATG GGCTAGCTACAACGA GTGAGATA 19	13
917	UCUCACAC A UCGACAAA	212	TTTGTCGA GGCTAGCTACAACGA GTGTGAGA 19	914
921	ACACAUCG A CAAACCAA	213		15
925	AUCGACAA A CCAAUACA	214		916
929	ACAAACCA A UACAAUCA	215		917
931	AAACCAAU A CAAUCAUA	216		918
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967	CACGCCCA G UCAAAUUA	228	<u> </u>	L930
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975	ACUUAGAG G CCAUACUC	230		L932
983	UAGAGGCC A UACUCUUG	231		1933
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988	AUACUCUU G UCCUCAAU	233		1935
994	UGUCCUCA A UUGUACUG	234		1936
1001	CCUCAAUU G UACUGCUA	235		1937
1004	UCAAUUGU A CUGCUACC	236		1938
1006	AUUGUACU G CUACCACU	237	والمراجعة والمناطقة والمناطة والمناطقة والمناطقة والمناطقة والمناطقة والمناطقة والمناطقة والمناطقة والمناطقة والمناط	1939
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1012	CUGCUACC A CUCCCUUG	239		1941
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1025	UCCCUUGA A CACGAGAGUU CCUUGAAC A CGAGAGUU	241		1943
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1042		244	CAGGGTAA GGCTAGCTACAACGA TCCAGGTC	1947
1049	GACCUGGA G UUACCCUG	245	CATCAGGG GGCTAGCTACAACGA AACTCCAG	1948
1052	CUGGAGUU A CCCUGAUG	247		1949
1058	UUACCCUG A UGAAAAAA	248	CTCTCTTA GGCTAGCTACAACGA TTTTTTCA	1950
1067		248	TACGGAAG GGCTAGCTACAACGA TCTCTTAT	1951
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1081		250	ATTCGTCG GGCTAGCTACAACGA CTTACGGA	1953
1086		251	TCAATTCG GGCTAGCTACAACGA CGCCTTAC	1954
1089		252	TCAATICG GGCTAGCTACAACGA CGCCTTAC TTGGTCAA GGCTAGCTACAACGA TCGTCGCC	1955
1093		253		1956
1097		254	TGCTTTGG GGCTAGCTACAACGA CAATTCGT	1957
1103		255	GGGAATTG GGCTAGCTACAACGA TTTGGTCA	
1106	CCAAAGCA A UUCCCAUG	256	CATGGGAA GGCTAGCTACAACGA TGCTTTGG	1930

		T	TGTTGGCA GGCTAGCTACAACGA GGGAATTG 1959
1112	CAAUUCCC A UGCCAACA		TATGTTGG GGCTAGCTACAACGA ATGGGAAT 1960
1114	AUUCCCAU G CCAACAUA		AGAATATG GGCTAGCTACAACGA TGGCATGG 1961
1118	CCAUGCCA A CAUAUUCU		GTAGAATA GGCTAGCTACAACGA GTTGGCAT 1962
1120	AUGCCAAC A UAUUCUAC		CTGTAGAA GGCTAGCTACAACGA ATGTTGGC 1963
1122	GCCAACAU A UUCUACAG		GAACACTG GGCTAGCTACAACGA AGAATATG 1964
1127	CAUAUUCU A CAGUGUUC		TAAGAACA GGCTAGCTACAACGA TGTAGAAT 1965
1130	AUUCUACA G UGUUCUUA	263	AGTAAGAA GGCTAGCTACAACGA ACTGTAGA 1966
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1197	AGGAGUGG A CCAUCAUU	279	AATGATGG GGCTAGCTACAACGA CCACTCCT 1981
1200	AGUGGACC A UCAUUCAA	280	TTGAATGA GGCTAGCTACAACGA GGTCCACT 1982
1203	GGACCAUC A UUCAAAUC	281	GATTTGAA GGCTAGCTACAACGA GATGGTCC 1983
1209	UCAUUCAA A UCUGUUAA	282	TTAACAGA GGCTAGCTACAACGA TTGAATGA 1984
1213	UCAAAUCU G UUAACACC	283	GGTGTTAA GGCTAGCTACAACGA AGATTTGA 1985
1217	AUCUGUUA A CACCUCAG	284	CTGAGGTG GGCTAGCTACAACGA TAACAGAT 1986
1219	CUGUUAAC A CCUCAGUG	285	CACTGAGG GGCTAGCTACAACGA GTTAACAG 1987
1225	ACACCUCA G UGCAUAUA	286	TATATGCA GGCTAGCTACAACGA TGAGGTGT 1988
1227	ACCUCAGU G CAUAUAUA	287	TATATATG GGCTAGCTACAACGA ACTGAGGT 1989
1229	CUCAGUGC A UAUAUAUG	288	CATATATA GGCTAGCTACAACGA GCACTGAG 1990
1231	CAGUGCAU A UAUAUGAU	289	ATCATATA GGCTAGCTACAACGA ATGCACTG 1991
1233	GUGCAUAU A UAUGAUAA	290	TTATCATA GGCTAGCTACAACGA ATATGCAC 1992
1235	GCAUAUAU A UGAUAAAG	291	CTTTATCA GGCTAGCTACAACGA ATATATGC 1993
1238	UAUAUAUG A UAAAGCAU	292	ATGCTTTA GGCTAGCTACAACGA CATATATA 1994
1243	AUGAUAAA G CAUUCAUC	293	GATGAATG GGCTAGCTACAACGA TTTATCAT 1995
1245	GAUAAAGC A UUCAUCAC	294	GTGATGAA GGCTAGCTACAACGA GCTTTATC 1996
1249	AAGCAUUC A UCACUGUG	295	CACAGTGA GGCTAGCTACAACGA GAATGCTT 1997
1252	CAUUCAUC A CUGUGAAA	296	TTTCACAG GGCTAGCTACAACGA GATGAATG 1998
1255	UCAUCACU G UGAAACAU	297	ATGTTTCA GGCTAGCTACAACGA AGTGATGA 1999
1260	ACUGUGAA A CAUCGAAA	298	TTTCGATG GGCTAGCTACAACGA TTCACAGT 2000
1262	UGUGAAAC A UCGAAAAC	299	GTTTTCGA GGCTAGCTACAACGA GTTTCACA 2001
1269	CAUCGAAA A CAGCAGGU	300	ACCTGCTG GGCTAGCTACAACGA TTTCGATG 2002
1272	CGAAAACA G CAGGUGCU	301	AGCACCTG GGCTAGCTACAACGA TGTTTTCG 2003
1276	AACAGCAG G UGCUUGAA	302	TTCAAGCA GGCTAGCTACAACGA CTGCTGTT 2004
1278	CAGCAGGU G CUUGAAAC	303	GTTTCAAG GGCTAGCTACAACGA ACCTGCTG 2005
1285	UGCUUGAA A CCGUAGCU	304	AGCTACGG GGCTAGCTACAACGA TTCAAGCA 2006
1288		305	GCCAGCTA GGCTAGCTACAACGA GGTTTCAA 2007
1291		306	CTTGCCAG GGCTAGCTACAACGA TACGGTTT 2008
1295		307	ACCGCTTG GGCTAGCTACAACGA CAGCTACG 2009
1299		308	TAAGACCG GGCTAGCTACAACGA TTGCCAGC 2010

1307 GCGANGGG G UCUUNCGG 309 CGGTANGA GGCTAGCTACACGA AGACCGC 2012 1311 UCUUNCGG G CUCUCUMU 311 ARTAGARG GGCTAGCTACACGA AGACCGC 2013 1311 CUCUNCGG G CUCUCUMU 311 ARTAGARG GGCTAGCTACACGA AGACGACC 2014 1312 CCATTTCA GGCTAGCTACACGA AGAGGAGC 2014 1312 CCATTTCA GGCTAGCTACACGA AGAGGAGCC 2014 1312 CCATTTCA GGCTAGCTACACGA AGAGGAGC 2014 1324 CURUGAGAGCA 313 GGCTAGCTACACGA AGAGGAGCC 2015 1329 GUGAAGGCA 314 GGGANATG GGCTAGCTACACGA TATCCATT 2015 1324 UUUCCCUC GCCGGAGGU 315 GAGGGAA GGCTAGCTACACGA TATCCATC 2017 1341 UUUCCCUC GCCGGAGGU 316 CATTCCGG GGCTAGCTACACGA TATCCAGGO 2019 1351 CGGAGGUU GUGUDAGG 317 CCATTCAG GGCTAGCTACACGA CACCTACCACGA 1351 CGGAGGUU GUGUDAA 318 TAACCATA GGCTAGCTACACGA ACACTTC 2021 1355 GGUGUAGAG GUGUDAGA 319 TAACCATA GGCTAGCTACACGA ACACTTC 2021 1356 GUUGUAGAG AUGUGUAC 311 TAACCATA GGCTAGCTACACGA ACACTTC 2021 1356 GUUGUAGAG GUUACAAGA 320 TCTTTTAA GGCTAGCTACAACGA CATACAAC 2022 1364 GUUACAGG GUUACAAGA 321 GTAACCC AGGCTAGCTACAACGA CATACAAC 2022 1364 GUUACAG GUUACAGGA 322 GTCACCAG GGCTAGCTACAACGA CATACAAC 2022 1375 GGUUACCU G CUACUAGA 322 GTCACAGG GGCTAGCTACAACGA CATACAAC 2024 1371 GAUGGGUU A CCUGCGAC 322 GTCACAGG GGCTAGCTACAACGA CATACAAC 2025 1375 GGUUACCU G CUACUGAG 324 CTCAGTCG GGCTAGCTACAACGA CATACAAC 2026 1376 UACCUGC G CUACUGAG 324 CTCAGTCG GGCTAGCTACAACGA CATACAAC 2027 1376 GUUACCU G CUACUGA 324 CTCAGTCG GGCTAGCTACAACGA CAGGTAC 2027 1376 UACCUGCC GUCACUGA 327 TTCTCAG GGCTAGCTACAACGA AGGTAAC 2027 1376 UACCUGCC GUCACUGA 327 TTCTCAG GGCTAGCTACAACGA AGGTAAC 2027 1376 UACCUGCC GUCACUGA 327 TTCTCAG GGCTAGCTACAACGA AGGTACC 2027 1379 UACCUGCC GUCACUGA 327 TTCTCAG GGCTAGCTACAACGA AGAATTCC 2027 1379 UACCUGCC GUCACUGA 327 TTCTCAG GGCTAGCTACAACGA AGAATTCC 2027 1379 UACCUGCC AUGUACAA 338 TCCATAG GGCTAGCTACAACGA AGAA				CCCTAAGA GGCTAGCTACAACGA CGCTTGCC 2011	_
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1396	1378	UACCUGCG A CUGAGAAA	325	TITCICAG GGCIAGGIAGIAGIAG	
1394	1386	ACUGAGAA A UCUGCUCG	326	COROCACA COCINOCATACA CARACTERISTA CARACTERI	
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1552	AAAAGGCC G UGUCAUCG	366	CGATGACA GGCTAGCTACAACGA GGCCTTTT	8905
1554	AAGGCCGU G UCAUCGUU	367	AACGATGA GGCTAGCTACAACGA ACGGCCTT	2069
1557	GCCGUGUC A UCGUUUCC	368	GGAAACGA GGCTAGCTACAACGA GACACGGC	2070
1560	GUGUCAUC G UUUCCAGA	369	TCTGGAAA GGCTAGCTACAACGA GATGACAC	2071
1568	GUUUCCAG A CCCGGCUC	370	GAGCCGGG GGCTAGCTACAACGA CTGGAAAC	2072
1573	CAGACCCG G CUCUCUAC	371	GTAGAGAG GGCTAGCTACAACGA CGGGTCTG	2073
1580	GGCUCUCU A CCCACUGG	372	CCAGTGGG GGCTAGCTACAACGA AGAGAGCC	2074
1584	CUCUACCC A CUGGGCAG	373	CTGCCCAG GGCTAGCTACAACGA GGGTAGAG	2075
1589	CCCACUGG G CAGCAGAC	374	GTCTGCTG GGCTAGCTACAACGA CCAGTGGG	2076
1592	ACUGGGCA G CAGACAAA	375	TTTGTCTG GGCTAGCTACAACGA TGCCCAGT	2077
1596	GGCAGCAG A CAAAUCCU	376	AGGATTTG GGCTAGCTACAACGA CTGCTGCC	2078
1600	GCAGACAA A UCCUGACU	377	AGTCAGGA GGCTAGCTACAACGA TTGTCTGC	2079
1606	AAAUCCUG A CUUGUACC	378	GGTACAAG GGCTAGCTACAACGA CAGGATTT	2080
1610	CCUGACUU G UACCGCAU	379	ATGCGGTA GGCTAGCTACAACGA AAGTCAGG	2081
1612	UGACUUGU A CCGCAUAU	380	ATATGCGG GGCTAGCTACAACGA ACAAGTCA	2082
1615	CUUGUACC G CAUAUGGU	381	ACCATATG GGCTAGCTACAACGA GGTACAAG	2083
1617	UGUACCGC A UAUGGUAU	382	ATACCATA GGCTAGCTACAACGA GCGGTACA	2084
1619	UACCGCAU A UGGUAUCC	383	GGATACCA GGCTAGCTACAACGA ATGCGGTA	2085
1622	CGCAUAUG G UAUCCCUC	384	GAGGGATA GGCTAGCTACAACGA CATATGCG	2086
1624	CAUAUGGU A UCCCUCAA	385	TTGAGGGA GGCTAGCTACAACGA ACCATATG	2087
1632	AUCCCUCA A CCUACAAU	386	ATTGTAGG GGCTAGCTACAACGA TGAGGGAT	2088
1636	. CUCAACCU A CAAUCAAG	387	CTTGATTG GGCTAGCTACAACGA AGGTTGAG	2089
1639	AACCUACA A UCAAGUGG	388	CCACTTGA GGCTAGCTACAACGA TGTAGGTT	2090
1644	ACAAUCAA G UGGUUCUG	389	CAGAACCA GGCTAGCTACAACGA TTGATTGT	2091
1647	AUCAAGUG G UUCUGGCA	390	TGCCAGAA GGCTAGCTACAACGA CACTTGAT	2092
1653	UGGUUCUG G CACCCCUG	391	CAGGGGTG GGCTAGCTACAACGA CAGAACCA	2093
1655	GUUCUGGC A CCCCUGUA	392	TACAGGGG GGCTAGCTACAACGA GCCAGAAC	2094
1661	GCACCCCU G UAACCAUA	393	TATGGTTA GGCTAGCTACAACGA AGGGGTGC	2095
1664	CCCCUGUA A CCAUAAUC	394	GATTATGG GGCTAGCTACAACGA TACAGGGG	2096
1667	CUGUAACC A UAAUCAUU	395	AATGATTA GGCTAGCTACAACGA GGTTACAG	2097
1670	UAACCAUA A UCAUUCCG	396	CGGAATGA GGCTAGCTACAACGA TATGGTTA	2098
1673	CCAUAAUC A UUCCGAAG	397	CTTCGGAA GGCTAGCTACAACGA GATTATGG	2099
1681	AUUCCGAA G CAAGGUGU	398	ACACCTTG GGCTAGCTACAACGA TTCGGAAT	2100
1686	GAAGCAAG G UGUGACUU	399	AAGTCACA GGCTAGCTACAACGA CTTGCTTC	2101
1688	AGCAAGGU G UGACUUUU	400	AAAAGTCA GGCTAGCTACAACGA ACCTTGCT	2102
1691		401	AACAAAAG GGCTAGCTACAACGA CACACCTT	2103
1697		402	TATTGGAA GGCTAGCTACAACGA AAAAGTCA	2104
1703	UUGUUCCA A UAAUGAAG	403	CTTCATTA GGCTAGCTACAACGA TGGAACAA	2105
1706		404	ACTOTTCA GGCTAGCTACAACGA TATTGGAA	
1713		405	ATAAAGGA GGCTAGCTACAACGA TCTTCATT	-
1720		406	ATCCAGGA GGCTAGCTACAACGA AAAGGACT	
1727		407	TGTCAGCA GGCTAGCTACAACGA CCAGGATA	
1729		408	GCTGTCAG GGCTAGCTACAACGA ATCCAGGA	
1733		409	TGTTGCTG GGCTAGCTACAACGA CAGCATCO	}
1736		410	CCATGTTG GGCTAGCTACAACGA TGTCAGC	
1739		411	TTCCCATG GGCTAGCTACAACGA TGCTGTC	
		412	GTTTCCCA GGCTAGCTACAACGA GTTGCTG	
1741	MUNDENAL A DOGGAMAC	7.2		

				
1748	CAUGGGAA A CAGAAUUG	413	CAATTCTG GGCTAGCTACAACGA TTCCCATG 2	115
1753	GAAACAGA A UUGAGAGC	414	GCTCTCAA GGCTAGCTACAACGA TCTGTTTC 2	116
1760	AAUUGAGA G CAUCACUC	415	GAGTGATG GGCTAGCTACAACGA TCTCAATT 2	117
1762	UUGAGAGC A UCACUCAG	416	CTGAGTGA GGCTAGCTACAACGA GCTCTCAA 2	118
1765	AGAGCAUC A CUCAGCGC	417	GCGCTGAG GGCTAGCTACAACGA GATGCTCT 2	119
1770	AUCACUCA G CGCAUGGC	418	GCCATGCG GGCTAGCTACAACGA TGAGTGAT 2	120
1772	CACUCAGO G CAUGGOAA	419	TTGCCATG GGCTAGCTACAACGA GCTGAGTG 2	121
1774	CUCAGCGC A UGGCAAUA	420	TATTGCCA GGCTAGCTACAACGA GCGCTGAG 2	122
1777	AGCGCAUG G CAAUAAUA	421	TATTATTG GGCTAGCTACAACGA CATGCGCT 2	2123
1780	GCAUGGCA A UAAUAGAA	422	TTCTATTA GGCTAGCTACAACGA TGCCATGC 2	2124
1783	UGGCAAUA A UAGAAGGA	423	TCCTTCTA GGCTAGCTACAACGA TATTGCCA	2125
1796	AGGAAAGA A UAAGAUGG	424	CCATCTTA GGCTAGCTACAACGA TCTTTCCT	2126
1801	AGAAUAAG A UGGCUAGC	425	GCTAGCCA GGCTAGCTACAACGA CTTATTCT	2127
1804	AUAAGAUG G CUAGCACC	426		2128
1808	GAUGGCUA G CACCUUGG	427		2129
1810	UGGCUAGC A CCUUGGUU	428		2130
1816	GCACCUUG G UUGUGGCU	429		2131
	CCUUGGUU G UGGCUGAC	430		2132
1819	UGGUUGUG G CUGACUCU	431		2133
1822		432		2134
1826	UGUGGCUG A CUCUAGAA	433		2135
1834	ACUCUAGA A UUUCUGGA	434	ICCAGAMA GGCIAGGIAGA	2136
1843	UUUCUGGA A UCUACAUU		MAIGIAGA GGGIAGGIAGA	2137
1847	UGGAAUCU A CAUUUGCA	435	IGCHIAIC GCCIIIGCIIICCI	2138
1849	GAAUCUAC A UUUGCAUA	436	AAGCTATG GGCTAGCTACAACGA AAATGTAG	
1853	CUACAUUU G CAUAGCUU	437	GGAAGCTA GGCTAGCTACAACGA GCAAATGT	
1855	ACAUUUGC A UAGCUUCC	438	ATTGGAAG GGCTAGCTACAACGA TATGCAAA	
1858	UUUGCAUA G CUUCCAAU	439	CAACTTTA GGCTAGCTACAACGA TGGAAGCT	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
1865	AGCUUCCA A UAAAGUUG	440	AGTCCCAA GGCTAGCTACAACGA TTTATTGG	
1870	CCAAUAAA G UUGGGACU	441		2144
1876	AAGUUGGG A CUGUGGGA	442	TCCCACAG GGCTAGCTACAACGA CCCAACTT	
1879	UUGGGACU G UGGGAAGA	443	TCTTCCCA GGCTAGCTACAACGA AGTCCCAA	
1889	GGGAAGAA A CAUAAGCU	444	AGCTTATG GGCTAGCTACAACGA TTCTTCCC	
1891	GAAGAAAC A UAAGCUUU	445	AAAGCTTA GGCTAGCTACAACGA GTTTCTTC	
1895	AAACAUAA G CUUUUAUA	446	TATAAAAG GGCTAGCTACAACGA TTATGTTT	
1901	AAGCUUUU A UAUCACAG	447	CTGTGATA GGCTAGCTACAACGA AAAAGCTT	
1903	GCUUUUAU A UCACAGAU	448	ATCTGTGA GGCTAGCTACAACGA ATAAAAGC	
1906	UUUAUAUC A CAGAUGUG	449	CACATCTG GGCTAGCTACAACGA GATATAAA	
1910	UAUCACAG A UGUGCCAA	450	TTGGCACA GGCTAGCTACAACGA CTGTGATA	
1912	UCACAGAU G UGCCAAAU	451	ATTTGGCA GGCTAGCTACAACGA ATCTGTGA	
1914	ACAGAUGU G CCAAAUGG	452	CCATTIGG GGCTAGCTACAACGA ACATCTGT	
1919	UGUGCCAA A UGGGUUUC	453	GAAACCCA GGCTAGCTACAACGA TTGGCACA	
1923	CCAAAUGG G UUUCAUGU	454	ACATGAAA GGCTAGCTACAACGA CCATTTGG	
1928	UGGGUUUC A UGUUAACU	455	AGTTAACA GGCTAGCTACAACGA GAAACCCA	
1930	GGUUUCAU G UUAACUUG	456	CAAGTTAA GGCTAGCTACAACGA ATGAAACC	
1934	UCAUGUUA A CUUGGAAA	457	TTTCCAAG GGCTAGCTACAACGA TAACATGA	
1945	UGGAAAAA A UGCCGACG	458	CGTCGGCA GGCTAGCTACAACGA TTTTTCCA	
1947	GAAAAAAU G CCGACGGA	459	TCCGTCGG GGCTAGCTACAACGA ATTTTTTC	
1951	AAAUGCCG A CGGAAGGA	460	TCCTTCCG GGCTAGCTACAACGA CGGCATTT	2162
1964		461	GTTTCAGG GGCTAGCTACAACGA CCTCTCCT	2163
1971		462	CAAGACAG GGCTAGCTACAACGA TTCAGGTC	2164
1974		463	GTGCAAGA GGCTAGCTACAACGA AGTTTCAG	2165
1979		464	TAACTGTG GGCTAGCTACAACGA AAGACAGT	2166

			CONTROL CONTRO
1981	UGUCUUGC A CAGUUAAC		GTTAACTG GGCTAGCTACAACGA GCAAGACA 2167
1984	CUUGCACA G UUAACAAG		CTTGTTAA GGCTAGCTACAACGA TGTGCAAG 2168
1988	CACAGUUA A CAAGUUCU		AGAACTTG GGCTAGCTACAACGA TAACTGTG 2169
1992	GUUAACAA G UUCUUAUA		TATAAGAA GGCTAGCTACAACGA TTGTTAAC 2170
1998	AAGUUCUU A UACAGAGA	469	TCTCTGTA GGCTAGCTACAACGA AAGAACTT 2171
2000	GUUCUUAU A CAGAGACG	470	CGTCTCTG GGCTAGCTACAACGA ATAAGAAC 2172
2006	AUACAGAG A CGUUACUU	471	AAGTAACG :GGCTAGCTACAACGA CTCTGTAT 2173
2008	ACAGAGAC G UUACUUGG	472	CCAAGTAA GGCTAGCTACAACGA GTCTCTGT 2174
2011	GAGACGUU A CUUGGAUU	473	AATCCAAG GGCTAGCTACAACGA AACGTCTC 2175
2017	UUACUUGG A UUUUACUG	474	CAGTAAAA GGCTAGCTACAACGA CCAAGTAA 2176
2022	UGGAUUUU A CUGCGGAC	475	GTCCGCAG GGCTAGCTACAACGA AAAATCCA 2177
2025	AUUUUACU G CGGACAGU	476	ACTGTCCG GGCTAGCTACAACGA AGTAAAAT 2178
2029	UACUGCGG A CAGUUAAU	477	ATTAACTG GGCTAGCTACAACGA CCGCAGTA 2179
2032	UGCGGACA G UUAAUAAC	478	GTTATTAA GGCTAGCTACAACGA TGTCCGCA 2180
2036	GACAGUUA A UAACAGAA	479	TTCTGTTA GGCTAGCTACAACGA TAACTGTC 2181
2039	AGUUAAUA A CAGAACAA	480	TTGTTCTG GGCTAGCTACAACGA TATTAACT 2182
2044	AUAACAGA A CAAUGCAC	481	GTGCATTG GGCTAGCTACAACGA TCTGTTAT 2183
2047	ACAGAACA A UGCACUAC	482	GTAGTGCA GGCTAGCTACAACGA TGTTCTGT 2184
2049	AGAACAAU G CACUACAG	483	CTGTAGTG GGCTAGCTACAACGA ATTGTTCT 2185
2051	AACAAUGC A CUACAGUA	484	TACTGTAG GGCTAGCTACAACGA GCATTGTT 2186
2054	AAUGCACU A CAGUAUUA	485	TAATACTG GGCTAGCTACAACGA AGTGCATT 2187
2057	GCACUACA G UAUUAGCA	486	TGCTAATA GGCTAGCTACAACGA TGTAGTGC 2188
2059	ACUACAGU A UUAGCAAG	487	CTTGCTAA GGCTAGCTACAACGA ACTGTAGT 2189
2063	CAGUAUUA G CAAGCAAA	488	TTTGCTTG GGCTAGCTACAACGA TAATACTG 2190
2067	AUUAGCAA G CAAAAAU	489	ATTTTTTG GGCTAGCTACAACGA TTGCTAAT 2191
2074	AGCAAAAA A UGGCCAUC	490	GATGGCCA GGCTAGCTACAACGA TTTTTGCT 2192
2077	AAAAAAUG G CCAUCACU	491	AGTGATGG GGCTAGCTACAACGA CATTTTTT 2193
2080	AAAUGGCC A UCACUAAG	492	CTTAGTGA GGCTAGCTACAACGA GGCCATTT 2194
2083	UGGCCAUC A CUAAGGAG	493	CTCCTTAG GGCTAGCTACAACGA GATGGCCA 2195
2091	ACUAAGGA G CACUCCAU	494	ATGGAGTG GGCTAGCTACAACGA TCCTTAGT 2196
2093	UAAGGAGC A CUCCAUCA	495	TGATGGAG GGCTAGCTACAACGA GCTCCTTA 2197
2098	AGCACUCC A UCACUCUU	496	AAGAGTGA GGCTAGCTACAACGA GGAGTGCT 2198
2101	ACUCCAUC A CUCUUAAU	497	ATTAAGAG GGCTAGCTACAACGA GATGGAGT 2199
2108	CACUCUUA A UCUUACCA	498	TGGTAAGA GGCTAGCTACAACGA TAAGAGTG 2200
2113	UUAAUCUU A CCAUCAUG	499	CATGATGG GGCTAGCTACAACGA AAGATTAA 2201
2116	AUCUUACC A UCAUGAAU	500	ATTCATGA GGCTAGCTACAACGA GGTAAGAT 2202
2119	UUACCAUC A UGAAUGUU	501	AACATTCA GGCTAGCTACAACGA GATGGTAA 2203
2123	CAUCAUGA A UGUUUCCC	502	GGGAAACA GGCTAGCTACAACGA TCATGATG 2204
2125	UCAUGAAU G UUUCCCUG	503	CAGGGAAA GGCTAGCTACAACGA ATTCATGA 2205
2133	GUUUCCCU G CAAGAUUC	504	GAATCTTG GGCTAGCTACAACGA AGGGAAAC 2206
2138	CCUGCAAG A UUCAGGCA	505	TGCCTGAA GGCTAGCTACAACGA CTTGCAGG 2207
2144	AGAUUCAG G CACCUAUG	506	CATAGGTG GGCTAGCTACAACGA CTGAATCT 2208
2146	AUUCAGGC A CCUAUGCC	507	GGCATAGG GGCTAGCTACAACGA GCCTGAAT 2209
2150	AGGCACCU A UGCCUGCA	508	TGCAGGCA GGCTAGCTACAACGA AGGTGCCT 2210
2152	GCACCUAU G CCUGCAGA	509	TCTGCAGG GGCTAGCTACAACGA ATAGGTGC 2211
2156		510	TGGCTCTG GGCTAGCTACAACGA AGGCATAG 2212
2161		511	ATTCCTGG GGCTAGCTACAACGA TCTGCAGG 2213
2168		512	TGTATACA GGCTAGCTACAACGA TCCTGGCT 2214
2170		513	TGTGTATA GGCTAGCTACAACGA ATTCCTGG 2215
2172		514	CCTGTGTA GGCTAGCTACAACGA ACATTCCT 2216
<u>-</u>		515	CCCCTGTG GGCTAGCTACAACGA ATACATTC 221
2174	GAAUGUAU A CACAGGGG	1 272	CCCCIGIO COCINCATORI IIIICIII III

			TOTAL COMP CAR COM IMPORTAGE 2210
2188	GGGAAGAA A UCCUCCAG		CTGGAGGA GGCTAGCTACAACGA TTCTTCCC 2219
2206	AGAAAGAA A UUACAAUC		GATTGTAA GGCTAGCTACAACGA TTCTTTCT 2220
2209	AAGAAAUU A CAAUCAGA		TCTGATTG GGCTAGCTACAACGA AATTTCTT 2221
2212	AAAUUACA A UCAGAGAU		ATCTCTGA GGCTAGCTACAACGA TGTAATTT 2222
2219	AAUCAGAG A UCAGGAAG	521	CTTCCTGA GGCTAGCTACAACGA CTCTGATT 2223
2227	AUCAGGAA G CACCAUAC	522	GTATGGTG GGCTAGCTACAACGA TTCCTGAT 2224
2229	CAGGAAGC A CCAUACCU	523	AGGTATGG GGCTAGCTACAACGA GCTTCCTG 2225
2232	GAAGCACC A UACCUCCU	524	AGGAGGTA GGCTAGCTACAACGA GGTGCTTC 2226
2234	AGCACCAU A CCUCCUGC	525	GCAGGAGG GGCTAGCTACAACGA ATGGTGCT 2227
2241	UACCUCCU G CGAAACCU	526	AGGTTTCG GGCTAGCTACAACGA AGGAGGTA 2228
2246	CCUGCGAA A CCUCAGUG	527	CACTGAGG GGCTAGCTACAACGA TTCGCAGG 2229
2252	AAACCUCA G UGAUCACA	528	TGTGATCA GGCTAGCTACAACGA TGAGGTTT 2230
2255	CCUCAGUG A UCACACAG	529	CTGTGTGA GGCTAGCTACAACGA CACTGAGG 2231
2258	CAGUGAUC A CACAGUGG	530	CCACTGTG GGCTAGCTACAACGA GATCACTG 2232
	GUGAUCAC A CAGUGGCC	531	GGCCACTG GGCTAGCTACAACGA GTGATCAC 2233
2260		532	GATGGCCA GGCTAGCTACAACGA TGTGTGAT 2234
2263	AUCACACA G UGGCCAUC	533	GCTGATGG GGCTAGCTACAACGA CACTGTGT 2235
2266	ACACAGUG G CCAUCAGC	534	ACTGCTGA GGCTAGCTACAACGA GGCCACTG 2236
2269	CAGUGGCC A UCAGCAGU		TGGAACTG GGCTAGCTACAACGA TGATGGCC 2237
2273	GGCCAUCA G CAGUUCCA	535 536	TGGTGGAA GGCTAGCTACAACGA TGCTGATG 2238
2276	CAUCAGCA G UUCCACCA		TAAAGTGG GGCTAGCTACAACGA GGAACTGC 2239
2281	GCAGUUCC A CCACUUUA	537	GTCTAAAG GGCTAGCTACAACGA GGTGGAAC 2240
2284	GUUCCACC A CUUUAGAC	538	CATGACAG GGCTAGCTACAACGA CTAAAGTG 2241
2291	CACUUUAG A CUGUCAUG	539	CATOACAG COCIAGOTAGE
2294	UUUAGACU G UCAUGCUA	540	INGCATCA GCCIRCOLICIALOCA TILEGRAPHICA
2297	AGACUGUC A UGCUAAUG	541	CRI IIION CCCIIICO
2299	ACUGUCAU G CUAAUGGU	542	ACCATTAG GOOTHGOTHGOTH
2303	UCAUGCUA A UGGUGUCC	543	General Commodition
2306	UGCUAAUG G UGUCCCCG	544	CGGGGACA GGCTAGCTACAACGA CATTAGCA 2246
2308	CUAAUGGU G UCCCCGAG	545	CTCGGGGA GGCTAGCTACAACGA ACCATTAG 2247
2316	GUCCCCGA G CCUCAGAU	546	ATCTGAGG GGCTAGCTACAACGA TCGGGGAC 2248
2323	AGCCUCAG A UCACUUGG	547	CCAAGTGA GGCTAGCTACAACGA CTGAGGCT 2249
2326	CUCAGAUC A CUUGGUUU	548	AAACCAAG GGCTAGCTACAACGA GATCTGAG 2250
2331	AUCACUUG G UUUAAAAA	549	TTTTTAAA GGCTAGCTACAACGA CAAGTGAT 2251
2339	GUUUAAAA A CAACCACA	550	TGTGGTTG GGCTAGCTACAACGA TTTTAAAC 2252
2342	UAAAAACA A CCACAAAA	551	TTTTGTGG GGCTAGCTACAACGA TGTTTTA 2253
2345	AAACAACC A CAAAAUAC	552	GTATTTTG GGCTAGCTACAACGA GGTTGTTT 2254
2350	ACCACAAA A UACAACAA	553	TTGTTGTA GGCTAGCTACAACGA TTTGTGGT 2255
2352	CACAAAAU A CAACAAGA	554	TCTTGTTG GGCTAGCTACAACGA ATTTTGTG 2256
2355	AAAAUACA A CAAGAGCC	555	GGCTCTTG GGCTAGCTACAACGA TGTATTTT 2257
2361	CAACAAGA G CCUGGAAU	556	ATTCCAGG GGCTAGCTACAACGA TCTTGTTG 2258
2368	AGCCUGGA A UUAUUUUA	557	TAAAATAA GGCTAGCTACAACGA TCCAGGCT 2259
2371	CUGGAAUU A UUUUAGGA	558	TCCTAAAA GGCTAGCTACAACGA AATTCCAG 2260
2379		559	CTTCCTGG GGCTAGCTACAACGA CCTAAAAT 2261
2387		560	GCGTGCTG GGCTAGCTACAACGA TTCCTGGT 2262
2390		561	ACAGCGTG GGCTAGCTACAACGA TGCTTCCT 2263
		562	AAACAGCG GGCTAGCTACAACGA GCTGCTTC 2264
2392		563	ATAAACAG GGCTAGCTACAACGA GTGCTGCT 2265
2394		564	TCAATAAA GGCTAGCTACAACGA AGCGTGCT 2266
2397		565	TCTTTCAA GGCTAGCTACAACGA AAACAGCG 2267
2401			TTCTGTGA GGCTAGCTACAACGA TCTTTCAA 2268
2410		566	CTCTTCTG GGCTAGCTACAACGA GACTCTTT 2269
2413		567	CICITOTO COCTINOTACIZACIONI
2423	AGAAGAGG A UGAAGGUG	568	CACCTTCA GGCTAGCTACAACGA CCTCTTCT 2270

			CATAGACA GGCTAGCTACAACGA CTTCATCC 2271
2429	GGAUGAAG G UGUCUAUC		GATAGACT COCTOCT
2431	AUGAAGGU G UCUAUCAC		GIGATACT COURT
2435	AGGUGUCU A UCACUGCA		IGCAOTOT COOLIGOTATION
2438	UGUCUAUC A CUGCAAAG		CITIONIC COOLINGIANCE
2441	CUAUCACU G CAAAGCCA		IGGC111G GGGTTGGTTGGTTGGTTGGTTGGTTGGTTGGTTGG
2446	ACUGCAAA G CCACCAAC		GTTGGTGG GGCTAGCTACAACGA TTTGCAGT 2276
2449	GCAAAGCC A CCAACCAG	575	CTGGTTGG GGCTAGCTACAACGA GGCTTTGC 2277
2453	AGCCACCA A CCAGAAGG	576	CCTTCTGG GGCTAGCTACAACGA TGGTGGCT 2278
2462	CCAGAAGG G CUCUGUGG	577	CCACAGAG GGCTAGCTACAACGA CCTTCTGG 2279
2467	AGGGCUCU G UGGAAAGU	578	ACTITCCA GGCTAGCTACAACGA AGAGCCCT 2280
2474	UGUGGAAA G UUCAGCAU	579	ATGCTGAA GGCTAGCTACAACGA TTTCCACA 2281
2479	AAAGUUCA G CAUACCUC	580	GAGGTATG GGCTAGCTACAACGA TGAACTTT 2282
2481	AGUUCAGC A UACCUCAC	581	GTGAGGTA GGCTAGCTACAACGA GCTGAACT 2283
2483	UUCAGCAU A CCUCACUG	582	CAGTGAGG GGCTAGCTACAACGA ATGCTGAA 2284
2488	CAUACCUC A CUGUUCAA	583	TTGAACAG GGCTAGCTACAACGA GAGGTATG 2285
2491	ACCUCACU G UUCAAGGA	584	TCCTTGAA GGCTAGCTACAACGA AGTGAGGT 2286
2500	UUCAAGGA A CCUCGGAC	585	GTCCGAGG GGCTAGCTACAACGA TCCTTGAA 2287
2507	AACCUCGG A CAAGUCUA	586	TAGACTTG GGCTAGCTACAACGA CCGAGGTT 2288
2511	UCGGACAA G UCUAAUCU	587	AGATTAGA GGCTAGCTACAACGA TTGTCCGA 2289
2516	CAAGUCUA A UCUGGAGC	588	GCTCCAGA GGCTAGCTACAACGA TAGACTTG 2290
2523	AAUCUGGA G CUGAUCAC	589	GTGATCAG GGCTAGCTACAACGA TCCAGATT 2291
2527	UGGAGCUG A UCACUCUA	590	TAGAGTGA GGCTAGCTACAACGA CAGCTCCA 2292
2530	AGCUGAUC A CUCUAACA	591	TGTTAGAG GGCTAGCTACAACGA GATCAGCT 2293
2536	UCACUCUA A CAUGCACC	592	GGTGCATG GGCTAGCTACAACGA TAGAGTGA 2294
2538	ACUCUAAC A UGCACCUG	593	CAGGTGCA GGCTAGCTACAACGA GTTAGAGT 2295
2540	UCUAACAU G CACCUGUG	594	CACAGGTG GGCTAGCTACAACGA ATGTTAGA 2296
2542	UAACAUGC A CCUGUGUG	595	CACACAGG GGCTAGCTACAACGA GCATGTTA 2297
2546	AUGCACCU G UGUGGCUG	596	CAGCCACA GGCTAGCTACAACGA AGGTGCAT 2298
2548	GCACCUGU G UGGCUGCG	597	CGCAGCCA GGCTAGCTACAACGA ACAGGTGC 2299
2551	CCUGUGUG G CUGCGACU	598	AGTCGCAG GGCTAGCTACAACGA CACACAGG 2300
2554	GUGUGGCU G CGACUCUC	599	GAGAGTCG GGCTAGCTACAACGA AGCCACAC 2301
2557	UGGCUGCG A CUCUCUUC	600	GAAGAGAG GGCTAGCTACAACGA CGCAGCCA 2302
2568	CUCUUCUG G CUCCUAUU	601	AATAGGAG GGCTAGCTACAACGA CAGAAGAG 2303
2574	UGGCUCCU A UUAACCCU	602	AGGGTTAA GGCTAGCTACAACGA AGGAGCCA 2304
2578	UCCUAUUA A CCCUCCUU	603	AAGGAGGG GGCTAGCTACAACGA TAATAGGA 2305
2587	CCCUCCUU A UCCGAAAA	604	TTTTCGGA GGCTAGCTACAACGA AAGGAGGG 2306
2596	UCCGAAAA A UGAAAAGG	605	CCTTTTCA GGCTAGCTACAACGA TTTTCGGA 2307
2604	AUGAAAAG G UCUUCUUC	606	GAAGAAGA GGCTAGCTACAACGA CTTTTCAT 2308
2617		607	AGTCTTTA GGCTAGCTACAACGA TTCAGAAG 2309
2623	AAAUAAAG A CUGACUAC	608	GTAGTCAG GGCTAGCTACAACGA CTTTATTT 2310
2627		609	ATAGGTAG GGCTAGCTACAACGA CAGTCTTT 2311
2630		610	TTGATAGG GGCTAGCTACAACGA AGTCAGTC 2312
2634		_}-	ATAATTGA GGCTAGCTACAACGA AGGTAGTC 2313
		612	CATTATAA GGCTAGCTACAACGA TGATAGGT 2314
2638		613	GTCCATTA GGCTAGCTACAACGA AATTGATA 2315
2641			TGGGTCCA GGCTAGCTACAACGA TATAATTG 2316
2644		615	CATCTGGG GGCTAGCTACAACGA CCATTATA 2317
2648			GAACTTCA GGCTAGCTACAACGA CTGGGTCC 2318
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2669			TCACACTG GGCTAGCTACAACGA TCATCCAA 2321
2673			CGCTCACA GGCTAGCTACAACGA TGCTCATC 2322
2676	GAUGAGCA G UGUGAGCG	620	COCICACA GGCIAGCIACAA IOCICAIC 2322

2678	UGAGCAGU G UGAGCGGC	621	GCCGCTCA GGCTAGCTACAACGA ACTGCTCA	2323
2682	CAGUGUGA G CGGCUCCC	622	GGGAGCCG GGCTAGCTACAACGA TCACACTG	2324
2685	UGUGAGCG G CUCCCUUA	623	TAAGGGAG GGCTAGCTACAACGA CGCTCACA	2325
2693	GCUCCCUU A UGAUGCCA	624	TGGCATCA GGCTAGCTACAACGA AAGGGAGC	2326
2696	CCCUUAUG A UGCCAGCA	625	TGCTGGCA GGCTAGCTACAACGA CATAAGGG	2327
2698	CUUAUGAU G CCAGCAAG	626	CTTGCTGG GGCTAGCTACAACGA ATCATAAG	2328
2702	UGAUGCCA G CAAGUGGG	627	CCCACTTG GGCTAGCTACAACGA TGGCATCA	2329 ·
2706	GCCAGCAA G UGGGAGUU	628	AACTCCCA GGCTAGCTACAACGA TTGCTGGC	2330
2712	AAGUGGGA G UUUGCCCG	629	CGGGCAAA GGCTAGCTACAACGA TCCCACTT	2331
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2733	AGACUUAA A CUGGGCAA	632	TTGCCCAG GGCTAGCTACAACGA TTAAGTCT	2334
2738	UAAACUGG G CAAAUCAC	633	GTGATTTG GGCTAGCTACAACGA CCAGTTTA	2335
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2758	GAAGAGGG G CUUUUGGA	636	TCCAAAAG GGCTAGCTACAACGA CCCTCTTC	2338
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2773	GAAAAGUG G UUCAAGCA	638	TGCTTGAA GGCTAGCTACAACGA CACTTTTC	2340
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2781	GUUCAAGC A UCAGCAUU	640	AATGCTGA GGCTAGCTACAACGA GCTTGAAC	2342
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2787	GCAUCAGC A UUUGGCAU	642	ATGCCAAA GGCTAGCTACAACGA GCTGATGC	2344
2792	AGCAUUUG G CAUUAAGA	643	TCTTAATG GGCTAGCTACAACGA CAAATGCT	2345
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2809	AAUCACCU A CGUGCCGG	647	CCGGCACG GGCTAGCTACAACGA AGGTGATT	2349
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2821	GCCGGACU G UGGCUGUG	651	CACAGCCA GGCTAGCTACAACGA AGTCCGGC	·····
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2909	CAUUGGCC A CCAUCUGA	671	TCAGATGG GGCTAGCTACAACGA GGCCAAT	
2912	UGGCCACC A UCUGAACG	672	CGTTCAGA GGCTAGCTACAACGA GGTGGCC	A 2374

, <u>.</u>			TAACCACG GGCTAGCTACAACGA TCAGATGG 2375
2918	CCAUCUGA A CGUGGUUA		CONTRACTOR CONTRACTOR CONTRACTOR
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2923	UGAACGUG G UUAACCUG		
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2931	GUUAACCU G CUGGGAGC		GCTCCCAO GCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
2938	UGCUGGGA G CCUGCACC		GGTGCAGG GGGTAGG GARA
2942	GGGAGCCU G CACCAAGC	679	GC11GG1G GCC11EG211G21GG1GG1GG1GG1GG1GG1GG1GG1GG1GG1GG1
2944	GAGCCUGC A CCAAGCAA	680	ligering decimentation
2949	UGCACCAA G CAAGGAGG	681	CCICCIIG COCINGO COCINGO COCI
2958	CAAGGAGG G CCUCUGAU	682	ATCAGAGG GGGTAGGTAGGTAGGTAGGTAGGTAGGTAGGTAG
2965	GGCCUCUG A UGGUGAUU	683	MATCHECH GOCINGONICATION
2968	CUCUGAUG G UGAUUGUU	684	AACANICA CCCIIICO
2971	UGAUGGUG A UUGUUGAA	685	TICARCAN GOOTHUTANA
2974	UGGUGAUU G UUGAAUAC	686	GTATTCAA GGCTAGCTACAACGA AATCACCA 2388
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3005	UCUCUCCA A CUACCUCA	693	TGAGGTAG GGCTAGCTACAACGA TGGAGAGA 2395
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3087	AAAAUGGA G CCAGGCCU	710	AGGCCTGG GGCTAGCTACAACGA TCCATTTT 2412
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3134		720	TTTCGCTG GGCTAGCTACAACGA TGGTGACG 2422
313		721	AGCTTTCG GGCTAGCTACAACGA TGCTGGTG 2423
314			TCGCAAAG GGCTAGCTACAACGA TTTCGCTG 2424
314			GGAGCTCG GGCTAGCTACAACGA AAAGCTTT 2425
315			TOTAL COMPANY CONTROL MCCCANNC 2426
313	2 2000000. 0 0000000		

3158 3170 3176	GAGCUCCG G CUUUCAGG UCAGGAAG A UAAAAGUC		CIGARAG COCINECTICALICAL COCI	27
3176	UCAGGAAG A UAAAAGUC	72 <i>6</i> 1/		128
			GACITIA COCINOCIACIONI COC	29
2102	AGAUAAAA G UCUGAGUG		CACTCACAT CCCTTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT	130
3182	AAGUCUGA G UGAUGUUG		Character occupantions remains	431
3185	UCUGAGUG A UGUUGAGG	729	CCICWWas againment assessment	432
3187	UGAGUGAU G UUGAGGAA	730	TICCICAR OCCIMUCIACIAN INC.	433
3203	AGAGGAGG A UUCUGACG	731	CGICAGAA GGGAAGGAAGGA GGAAGGA Q	434
3209	GGAUUCUG A CGGUUUCU	732	AGAMACCO COCIMOCIALIZATION	435
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3218	CGGUUUCU A CAAGGAGC	734	GCICCITO CCCIMOCIMORIZACIO	437
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			0450	
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3620	UCCUGAGU A CUCUACUC	816	GAGIAGAG GGC::GG::GG	518
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3668		827	CTTTTGGG GGCTAGCTACAACGA CTCTGTGC 2	529
368:			AATCTTGG GGCTAGCTACAACGA CTTTCTTT 2	530
300.				

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3696	UUUGCAGA A CUUGUGGA		ICCACATO GODINESTA DE LA COMPONIO DE LA
3700	CAGAACUU G UGGAAAAA		TITITUM GOOTMOOTHER TO THE TOTAL STORY
3708	GUGGAAAA A CUAGGUGA		ICACCIAG CCCIACCIA
3713	AAAACUAG G UGAUUUGC	834	GCAANICA GGGINGG
3716	ACUAGGUG A UUUGCUUC	835	GAAGGAA COOLINGTIAN COOLINGTIA
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3727	UGCUUCAA G CAAAUGUA	837	TACATITO COCTADORACIONES DE 40
3731	UCAAGCAA A UGUACAAC	838	GIIGIACA GCCIAGOC
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3800	GUUUACAU A CUCAACUC	858	GAGTTGAG GGCTAGCTACAACGA ATGTAAAC 2560
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3839	CAAGGAAA G UAUUUCAG	862	CTGAAATA GGCTAGCTACAACGA TTTCCTTG 2564
3841		863	AGCTGAAA GGCTAGCTACAACGA ACTTTCCT 2565
3847		864	CTTCGGAG GGCTAGCTACAACGA TGAAATAC 2566
3855		865	GAATTAAA GGCTAGCTACAACGA TTCGGAGC 2567
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3869		867	CATCAGAG GGCTAGCTACAACGA TTCCTGAA 2569
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3878		869	ATCTGACA GGCTAGCTACAACGA CATCAGAG 2571
3880		870	ATATCTGA GGCTAGCTACAACGA ATCATCAG 2572
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3889		873	AGCATTTA GGCTAGCTACAACGA ATATCTGA 2575
3893		874	TGAAAGCA GGCTAGCTACAACGA TTACATAT 2576
3893		875	CTTGAAAG GGCTAGCTACAACGA ATTTACAT 2577
390			CTCATGAA GGCTAGCTACAACGA TTGAAAGC 2578
			CAGGCTCA GGCTAGCTACAACGA GAACTTGA 2579
390			TTTCCAGG GGCTAGCTACAACGA TCATGAAC 2580
391			GGTTTTGA GGCTAGCTACAACGA TCTTTCCA 2581
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392	B GAAUCAAA A CCUUUGAA	880	

			CONTRACTOR COMPAGNACION MONTON NA 20	83
3939	UUUGAAGA A CUUUUACC		GGIMATA CCCI.	584
3945	GAACUUUU A CCGAAUGC		CONTINUE CON	
3950	UUUACCGA A UGCCACCU	883		585
3952	UACCGAAU G CCACCUCC	884	30130200 3001	586
3955	CGAAUGCC A CCUCCAUG	885	CATGORDO COCINEDES COMO COMO COMO COMO COMO COMO COMO COM	587
3961	CCACCUCC A UGUUUGAU	886	ALCHARACT COOLINGTIAN CO.	
3963	ACCUCCAU G UUUGAUGA	887		589
3968	CAUGUUUG A UGACUACC	888	ODINOICH: COCINDONIA	590 591
3971	GUUUGAUG A CUACCAGG	889	CCIOOIAN COOLANDOL	
3974	UGAUGACU A CCAGGGCG	890		592
3980	CUACCAGG G CGACAGCA	891	10010100 0001110011001	593
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3989	CGACAGCA G CACUCUGU	894	ACRONO10 COULDED TO THE PROPERTY OF THE PROPER	596
3991	ACAGCAGC A CUCUGUUG	895	CHICAGAS COSTITUCIONES	2597
3996	AGCACUCU G UUGGCCUC	896	GAGGCCAA COCCIOCATION	2598
4000	CUCUGUUG G CCUCUCCC	897	GGGAGAGG GGC111GC111GC1	2599
4009	CCUCUCCC A UGCUGAAG	898	CITCHOCK COCINECTICAL COCINECTI	2600
4011	UCUCCCAU G CUGAAGCG	899	CGCITCHG GCCIAGCIIIC COM	2601
4017	AUGCUGAA G CGCUUCAC	900	GIGHNOCO GCCINGCING	2602
4019	GCUGAAGC G CUUCACCU	901	AGGTGGAG GGGTAGGTTTTTTTTTTTTTTTTTTTTTTT	2603
4024	AGCGCUUC A CCUGGACU	902	AGICANG GGCINGCINETICS TO THE	2604
4030	UCACCUGG A CUGACAGC	903	GCTGTCAG GGGZAGGZAGG	2605
4034	CUGGACUG A CAGCAAAC	904	G1116C16 G65116G114G11	2606 .
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4053	AAGGCCUC G CUCAAGAU	908	AICTIGAG GOCTIGGTIGGTIGGT	2610
4060	CGCUCAAG A UUGACUUG	909	CANOT CAN COCTION TO THE CANOT	2611
4064	CAAGAUUG A CUUGAGAG	910	CICICIDIO COCCINECTION	2612 2613
4072	ACUUGAGA G UAACCAGU	911	ACTGGTTA GGCTAGCTACAACGA TCTCAAGT	2614
4075	UGAGAGUA A CCAGUAAA	912	TTTACTGG GGCTAGCTACAACGA TACTCTCA	2615
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4151	GCACGUCA G CGAAGGCA	930	TGCCTTCG GGCTAGCTACAACGA TGACGTGC	
4157	CAGCGAAG G CAAGCGCA	931	TGCGCTTG GGCTAGCTACAACGA CTTCGCTG	1
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4252	UGUACUCC A CCCCACCC	955	TAGATGGG GGCTAGCTACAACGA GGGGTGGA 2657
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4379	CCAUGGGA G CCAGCUGC		GCAGCTGG GGCTAGCTACAACGA TCCCATGG 2687
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4596		1030	TTGGGTGA GGCTAGCTACAACGA CAGTGCAG 2732
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4608		1034	GTACGTGA GGCTAGCTACAACGA GCATTGGG 2736
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5 CUAGUAAG A UGCACUGA	1130	TCAGTGCA GGCTAGCTACAACGA CTTACTAG 2832
7 AGUAAGAU G CACUGAAA	1131	TTTCAGTG GGCTAGCTACAACGA ATCTTACT 2833
9 UAAGAUGC A CUGAAAAC	1132	GTTTTCAG GGCTAGCTACAACGA GCATCTTA 2834
6 CACUGAAA A CUUAGCCA	1133	TGGCTAAG GGCTAGCTACAACGA TTTCAGTG 2835
1 AAAACUUA G CCAGAGUU	1134	AACTCTGG GGCTAGCTACAACGA TAAGTTTT 2836
	1135	CAACCTAA GGCTAGCTACAACGA TCTGGCTA 2837
	1136	GGAGACAA GGCTAGCTACAACGA CTAACTCT 2838
	1137	CCTGGAGA GGCTAGCTACAACGA AACCTAAC 2839
		CATCATGG GGCTAGCTACAACGA CTGGAGAC 2840
6 UCCAGGCC A UGAUGGCC		GGCCATCA GGCTAGCTACAACGA GGCCTGGA 2841
		TAAGGCCA GGCTAGCTACAACGA CATGGCCT 2842
	AGGAGCAG A UGGACAGC GCAGAUGG A CAGCGAUG GAUGGACA G CGAUGAGG GGACAGCG A UGAGGGGA AUGAGGGGA A UUUUCUGG GAGGGGAC A UUUUCUGG UUUUCUGG A UUCUGGGA UCUGGGAG G CAAGAAAA AGAAAAGG A CAAAUAUC AAGGACAA A UAUCUUUU GGACAAAU A UCUUUUUU GAAGCAA A UUUUAGAC AAUUUUAGA A CCUUUACC AGACCUUU A CCUUUACC AGACCUUU A CCUUUACC AGACCUUU A UGGAAGUG CUUUACCU A UGGAAGUG CUUUACCU A UGGAAGUG GUUCUAU G UCCAUUCU GGGUUCU A UUCUCAUU GGGUUCU A UUCUCAUU CUAUGGA G UUCUAUGU CUAUGGC A UUCUCAUU CUAUGGC A UUCUCAUU CCAUUCUC A UUCGAGC UUCGAGG G CAUGUUUU CCAUUCU A UGGAAGUG CUUUGAUU G UGGCAUGU CCAUUCUC A UUCGAGC UUUGAUUU G UAGCACUG CAUUCUC A UUCGAGC UUUGAUUU G UAGCACUG CAUUCUC A UUCGAGC UUUGAUUU G UAGCACUG CAUUCUC A UUUGAACC CAUUCGG G CAUGUUUU CCAUUCGG G CAUGUUUU CCAUUCGG G CAUGUUUU CCAUUCGG G CAUGUUUU CCAUUCUC A UUUGAACC CAUUCGG G CAUGUUUU CUCAUUC G UGGCAUGU CUUGAGG G UGGCACUC AUGUGGGC A CUCAACC CGAGGGGG G CACUCAAC CGAGGGGG G CACUCAAC CACUGAGG G UGGCACUC CACUGAAA A CUUUGGC CACUGAAA A CUUCGAGC CUAGGGCC A UACUUUUG CACUCUCA G CACUGAAA CUAGAGGU G CACUGAAA CUAGAGGU G CACUGAAA CUAGAGGU G CACUCAAC CACUGAAA A CUUAGCCA CACUGAAA A CUCACCACAC CACUGAAA A CUUAGCCA CACUGAAA A CUUAGCACAC CACUGAAA A CUCACCACACC CACUGAAAA A CUUAGCACAC CACUGAAAA A CUUAGCACAC CACUGAAAA	AGGAGCAS A UGGACAGC 1090 GCAGAUGG A CAGCGAUG 1091 CCAUUGGACAG CGAUGAGG 1092 CGACAGCG A UGAGAGG 1093 TAUGAGAGG A UGAGAGGA 1093 TAUGAGAGGA A UGAGAGGA 1093 TAUGAGAGGA A UUUUUCU 1094 A CAGAGAGA A UUUUUCUGG 1095 CCAAGAAAA 1097 TAGAAAAAGGA A UUUUUUUU 1099 AAAGAAAAGGA A UAUUUUUU 1099 AAAGAAAAGGA A UAUUUUUUU 1099 AAAGAAAAA A UAUUUUUUU 1100 AAAGAAAAA CCAAAUUUU 1102 AAAUUUUAGA ACUUUAAAA CCUUUAAGA 1103 CUUUAAAAGAA A UUUUAAAACA 1101 TAGAAAAAGA A UUUUAAAACA 1100 AAAUUUUAAAA CCUUUAAAA CCUUUAAAA CCUUUAAAA CCUUUAAAAAAAA

			CHICHDAGG CGCTACTACTACGA CATCATGG 2843
5152	CCAUGAUG G CCUUACAC		GIGIAAGG GGCIAGCIAGCIAGCA DOGGCAM DOGG
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5166	CACUGAAA A UGUCACAU		ATGIGACA COCIACOTA CON A COMPANION COLOR
5168	CUGAAAAU G UCACAUUC		GAATGTGA GGCTAGCTACAACGA ATTTTCAG 2847
5171	AAAAUGUC A CAUUCUAU		AIAGAAIG GCCIAGGIAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
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5178	CACAUUCU A UUUUGGGU	1148	ACCCAAAA GGCTAGCTACAACGA AGAATGTG 2850
5185	UAUUUUGG G UAUUAAUA	1149	TATTAATA GGCTAGCTACAACGA CCAAAATA 2851
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5191	GGGUAUUA A UAUAUAGU	1151	ACTATATA GGCTAGCTACAACGA TAATACCC 2853
5193	GUAUUAAU A UAUAGUCC	1152	GGACTATA GGCTAGCTACAACGA ATTAATAC 2854
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5216	UUAACUCA A UUUCUUGG	1158	CCAAGAAA GGCTAGCTACAACGA TGAGTTAA 2860
5224	AUUUCUUG G UAUUAUUC	1159	GAATAATA GGCTAGCTACAACGA CAAGAAAT 2861
5226	UUCUUGGU A UUAUUCUG	1160	CAGAATAA GGCTAGCTACAACGA ACCAAGAA 2862
5229	UUGGUAUU A UUCUGUUU	1161	AAACAGAA GGCTAGCTACAACGA AATACCAA 2863
5234	AUUAUUCU G UUUUGCAC	1162	GTGCAAAA GGCTAGCTACAACGA AGAATAAT 2864
5239	UCUGUUUU G CACAGUUA	1163	TAACTGTG GGCTAGCTACAACGA AAAACAGA 2865
5241	UGUUUUGC A CAGUUAGU	1164	ACTAACTG GGCTAGCTACAACGA GCAAAACA 2866
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5251	AGUUAGUU G UGAAAGAA	1167	TTCTTTCA GGCTAGCTACAACGA AACTAACT 2869
5261	GAAAGAAA G CUGAGAAG	1168	CTTCTCAG GGCTAGCTACAACGA TTTCTTTC 2870
5271	UGAGAAGA A UGAAAAUG	1169	CATTTCA GGCTAGCTACAACGA TCTTCTCA 2871
5277	GAAUGAAA A UGCAGUCC	1170	GGACTGCA GGCTAGCTACAACGA TTTCATTC 2872
5279	AUGAAAAU G CAGUCCUG	1171	CAGGACTG GGCTAGCTACAACGA ATTTTCAT 2873
5282	AAAAUGCA G UCCUGAGG	1172	CCTCAGGA GGCTAGCTACAACGA TGCATTTT 2874
5294	UGAGGAGA G UUUUCUCC	1173	GGAGAAAA GGCTAGCTACAACGA TCTCCTCA 2875
5303	UUUUCUCC A UAUCAAAA	1174	TTTTGATA GGCTAGCTACAACGA GGAGAAAA 2876
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<u> </u>	AUAUCAAA A CGAGGGCU	1176	AGCCCTCG GGCTAGCTACAACGA TTTGATAT 2878
5311		1177	TCCATCAG GGCTAGCTACAACGA CCTCGTTT 2879
<u> </u>	GAGGGCUG A UGGAGGAA	1178	TTCCTCCA GGCTAGCTACAACGA CAGCCCTC 2880
5321		1179	CTTATTGA GGCTAGCTACAACGA CTTTTTCC 2881
5334		1180	TGACCTTA GGCTAGCTACAACGA TGACCTTT 2882
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5359		1184	GGTTGGTA GGCTAGCTACAACGA AGAGACGG 2886
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5371		1186	TGAATTGG GGCTAGCTACAACGA TTGGTTGG 2889
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5380		1188	TGTGTTGG GGCTAGCTACAACGA GAATTGGT 2891
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			CTTTTGGG GGCTAGCTACAACGA CCCAACTG 28	95
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5461	UAUCUCAC A CUAAUCUG	1206	CHORITAG GGGZZZGGZZZGGZ	908
5465	UCACACUA A UCUGAAAG	1207	CIIICAGA GGGIAGEIGEIGEIGEIGEIGEIGEIGEIGEIGEIGEIGEIGEIG	909
5475	CUGAAAGG A UGUGGAAG	1208	CIICCACA COCIACOLIA	910
5477	GAAAGGAU G UGGAAGAG	1209	CICIICAL OCCINGUING	911
5485	GUGGAAGA G CAUUAGCU	1210	AGCIANIC CCCITACCITACCITACCITACCITACCITACCITAC	2912
5487	GGAAGAGC A UUAGCUGG	1211	CCAGCIAN COCINOCIAL	2913
5491	GAGCAUUA G CUGGCGCA	1212	Ideacad coornage	2914
5495	AUUAGCUG G CGCAUAUU	1213	AMINIOCO COCIIDOINALIO	2915
5497	UAGCUGGC G CAUAUUAA	1214	TIMINIG GOCTIOCTION	2916
5499	GCUGGCGC A UAUUAAGC	1215	GCTIANTA GGCTAGGTTGTTGTT	2917
5501	UGGCGCAU A UUAAGCAC	1216	GIGCIIM GOOMSOOM	2918
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5508	UAUUAAGC A CUUUAAGC	1218	GCTTAAAG GGCTAGGTTGGTT	2920
5515	CACUUUAA G CUCCUUGA	1219	1CAAGGAG GGC17GG11GG11GG1	2921
5524	CUCCUUGA G UAAAAAGG	1220	CCITIIN OCCINODINATION I	2922
5532	GUAAAAG G UGGUAUGU	1221	ACATACCA GGCTAGCTACAACGA CTTTTTAC	2923
5535	AAAAGGUG G UAUGUAAU	1222	ATTACATA GGCTAGCTACAACGA CACCTTTT	2924
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5539	GGUGGUAU G UAAUUUAU	1224	ATAAATTA GGCTAGCTACAACGA ATACCACC	2926
5542	GGUAUGUA A UUUAUGCA	1225	TGCATAAA GGCTAGCTACAACGA TACATACC	2927
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5548	UAAUUUAU G CAAGGUAU	1227	ATACCTTG GGCTAGCTACAACGA ATAAATTA	2929
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5564	UUUCUCCA G UUGGGACU	1230	AGTCCCAA GGCTAGCTACAACGA TGGAGAAA	2932
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5577	GACUCAGG A UAUUAGUU	1232	AACTAATA GGCTAGCTACAACGA CCTGAGTC	2934
5579	CUCAGGAU A UUAGUUAA	1233	TTAACTAA GGCTAGCTACAACGA ATCCTGAG	2935
5583	GGAUAUUA G UUAAUGAG	1234	CTCATTAA GGCTAGCTACAACGA TAATATCC	2936
5587	AUUAGUUA A UGAGCCAU	1235	ATGGCTCA GGCTAGCTACAACGA TAACTAAT	
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5594	AAUGAGCC A UCACUAGA	1237	TCTAGTGA GGCTAGCTACAACGA GGCTCATT	
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5609	GAAGAAAA G CCCAUUUU	1239	AAAATGGG GGCTAGCTACAACGA TTTTCTTC	2941
5613	AAAAGCCC A UUUUCAAC	1240	GTTGAAAA GGCTAGCTACAACGA GGGCTTTT	2942
5620	CAUUUUCA A CUGCUUUG	1241	CAAAGCAG GGCTAGCTACAACGA TGAAAATG	2943
5623		1242	TTTCAAAG GGCTAGCTACAACGA AGTTGAAA	2944
5631		1243	CAGGCAAG GGCTAGCTACAACGA TTCAAAGC	2945

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5667	AUAGGGAG A CAGGGUAG	1250	CIACCETO COSTRUCTORIO	52
5672	GAGACAGG G UAGGAAAG	1251	CITICOTA GOCINOCITATION	53
5682	AGGAAAGG G CGCCUACU	1252	AGIAGOCG GOGIAGOZIA	54
5684	GAAAGGGC G CCUACUCU	1253	MINGINGO OCCUMENTATION OCCUPANTION OCCUPAN	955
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5698	UCUUCAGG G UCUAAAGA	1255	ICII INGN GOGLAGOZII.	957
5706	GUCUAAAG A UCAAGUGG	1256	CCACIION OCCINOCIICATION	958
5711	AAGAUCAA G UGGGCCUU	1257	Andocech Cournollia	959
5715	UCAAGUGG G CCUUGGAU	1258	AICCAAGG GGGIIGGIIGG	960
5722	GGCCUUGG A UCGCUAAG	1259	CITAGGA GCCIAGGILLET	961
5725	CUUGGAUC G CUAAGCUG	1260	CAUCITAG GGCIAGCIIIGITIGE	962
5730	AUCGCUAA G CUGGCUCU	1261	AGAGCCAG GGCTAGGTAGGTAGGT	963
5734	CUAAGCUG G CUCUGUUU	1262	AMACAGAG GGCIAGGIIGGI	964
5739	CUGGCUCU G UUUGAUGC	1263	GCATCAAA GGCTAGGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAG	965
5744	UCUGUUUG A UGCUAUUU	1264	AAATAGCA GGCTAGCTACAACGA CAAACAGA 2	966
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5749	UUGAUGCU A UUUAUGCA	1266	TGCATAAA GGCTAGCTACAACGA AGCATCAA 2	968
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5771	GGGUCUAU G UAUUUAGG	1272	CCTAAATA GGCTAGCTACAACGA ATAGACCC	2974
5773	GUCUAUGU A UUUAGGAU	1273	ATCCTAAA GGCTAGCTACAACGA ACATAGAC	2975
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5815	UCAAGUGG G CCUUGGAU	1281	ATCCAAGG GGCTAGCTACAACGA CCACTTGA	2983
5822	GGCCUUGG A UCGCUAAG	1282	CTTAGCGA GGCTAGCTACAACGA CCAAGGCC	2984
5825	CUUGGAUC G CUAAGCUG	1283	CAGCTTAG GGCTAGCTACAACGA GATCCAAG	2985
5830		1284	AGAGCCAG GGCTAGCTACAACGA TTAGCGAT	2986
5834		1285	AAACAGAG GGCTAGCTACAACGA CAGCTTAG	2987
5839		1286	GCATCAAA GGCTAGCTACAACGA AGAGCCAG	2988
5844		1287	AAATAGCA GGCTAGCTACAACGA CAAACAGA	2989
5846		1288	ATAAATAG GGCTAGCTACAACGA ATCAAACA	
5849		1289	TGCATAAA GGCTAGCTACAACGA AGCATCAA	2991
5853		1290	AACTTGCA GGCTAGCTACAACGA AAATAGCA	2992
5855		1291	CTAACTTG GGCTAGCTACAACGA ATAAATAG	2993
5859		1292	GACCCTAA GGCTAGCTACAACGA TTGCATAA	2994
5865		1293	TACATAGA GGCTAGCTACAACGA CCTAACTT	2995
		1294		2996
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6164	GUGGGACA G UCCUCUCC	1358	GGAGAGGA GGCTAGCTACAACGA TGTCCCAC 3060
6173	UCCUCUCC A CCAAGAUC	1359	GATCTTGG GGCTAGCTACAACGA GGAGAGGA 3061
6179	CCACCAAG A UCUAAAUC	1360	GATTTAGA GGCTAGCTACAACGA CTTGGTGG 3062
6185	AGAUCUAA A UCCAAACA	1361	TGTTTGGA GGCTAGCTACAACGA TTAGATCT 3063
6191	AAAUCCAA A CAAAAGCA	1362	TGCTTTTG GGCTAGCTACAACGA TTGGATTT 3064
6197	AAACAAAA G CAGGCUAG	1363	CTAGCCTG GGCTAGCTACAACGA TTTTGTTT 3065
6201	AAAAGCAG G CUAGAGCC	1364	GGCTCTAG GGCTAGCTACAACGA CTGCTTTT 3066
6207	AGGCUAGA G CCAGAAGA	1365	TCTTCTGG GGCTAGCTACAACGA TCTAGCCT 3067
6220	AAGAGAGG A CAAAUCUU	1366	AAGATTTG GGCTAGCTACAACGA CCTCTCTT 3068
6224	GAGGACAA A UCUUUGUU	1367	AACAAAGA GGCTAGCTACAACGA TTGTCCTC 3069
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6250	CUUUACAC A UACGCAAA	1372	TTTGCGTA GGCTAGCTACAACGA GTGTAAAG 3074
6252	UUACACAU A CGCAAACC	1373	GGTTTGCG GGCTAGCTACAACGA ATGTGTAA 3075
6254	ACACAUAC G CAAACCAC	1374	GTGGTTTG GGCTAGCTACAACGA GTATGTGT 3076
6258	AUACGCAA A CCACCUGU	1375	ACAGGTGG GGCTAGCTACAACGA TTGCGTAT 3077
6261	CGCAAACC A CCUGUGAC	1376	GTCACAGG GGCTAGCTACAACGA GGTTTGCG 3078
6265	AACCACCU G UGACAGCU	1377	AGCTGTCA GGCTAGCTACAACGA AGGTGGTT 3079
6268	CACCUGUG A CAGCUGGC	1378	GCCAGCTG GGCTAGCTACAACGA CACAGGTG 3080
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6459	AGACCAUA A UAAAUACU	1415		17
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6532	UCAGGUUU G UAGCAUAC	1427	GTATGCTA GGCTAGCTAGTTGGT	130
6535	GGUUUGUA G CAUACAUG	1428	CAIGIAIG GGCIACCIACLICAT	131
6537	UUUGUAGC A UACAUGAG	1429	CICAIGIA GGCIAGCIAGE	132
6539	UGUAGCAU A CAUGAGUC	1430	GACTCATG GGCTAGCTAGGT.GGT.	3133
6541	UAGCAUAC A UGAGUCCA	1431	TEGACICA GECTAGCIAGATEGAT	3134
6545	AUACAUGA G UCCAUCCA	1432	TGGATGGA GGCTAGCTAGATTGGT	3135
6549	AUGAGUCC A UCCAUCAG	1433	CIGATGGA GGCTAGCTACASTOCK	
6553	GUCCAUCC A UCAGUCAA	1434	TIGACIGA GGCIAGCIAGCIAGCIAGCIAGCIAGCIAGCIAGCIAGCI	3136
6557	AUCCAUCA G UCAAAGAA	1435	TICITION GGCINGCINGING	3137
6565	GUCAAAGA A UGGUUCCA	1436	TGGAACCA GGCTAGCTAGA	3138
6568	AAAGAAUG G UUCCAUCU	1437	AGAIGGAA GGCIAGCIACAIGGI GIO	3139
6573	AUGGUUCC A UCUGGAGU	1438	ACICCAGA GGCIAGCIACIZCO	3140
6580		1439	CATTANGA GGCTAGCTAGCTAG	3141
6586		1440	TITUTACA GGCTAGCTACATOO!	3142
6588		1441	TCTTCTA GGCTAGGTTAGTTGGT	3143
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6776	UACGUGGA A CAGUCUGG	1485	CCNONCIO COCIIDOTIA	3187
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6795	GGAAUGGG G CUGAAACC	1489	GG112G10 GGG11GG11GG	3191
6801	GGGCUGAA A CCAUGUGC	1490	OCHOLICO GOGILIOTEIGE	3192
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6818	AAGUCUGU G UCUUGUCA	1496	IGNORALIA GOOMINGOMINE	3198
6823	UGUGUCUU G UCAGUCCA	1497	16010101	3199
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6841		1501	CATCTCGG GGCTAGCTACAACGA GTCACTTC	3203
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6994	GUGUGUGU G UGUGUGUG	1542	CACACACA GGCTAGCTACAACGA ACACACAC	3244
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7026	GUGGGUGU A UGUGUGUU	1556	AACACACA GGCTAGCTACAACGA ACACCCAC	3258

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7028	GGGUGUAU G UGUGUUUU		ANACACA COCINECTION	259
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7206		1604	ATTACAAA GGCTAGCTACAACGA CCCAATTA	3306
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7547 GUALAGUI A CUCAUCACC 1675 GGTGATGG GGCTAGCTACAACGA CAAATATA 3377 7552 UAUAUUUGA A CCAUCACC 1675 GGTGATGG GGCTAGCTACAACGA GGTCAAAT 3378 7558 UGACCAUC A CCCUAUGG 1677 CCATAGGG GGCTAGCTACAACGA GGTCAAAT 3379 7558 UGACCAUC A CCCUAUGG 1677 CCATAGGG GGCTAGCTACAACGA GGTGATA 3380 7563 AUCACCCU A UGGAUAUU 1678 AATATCCA GGCTAGCTACAACGA AGGGTGAT 3380 7567 CCCUAUGG A UAUUGGCU 1679 AGCCAATA GGCTAGCTACAACGA CCATAGGG 3381 7569 CUAUGGAU A UUGGCUAG 1680 CTAGCCAA GGCTAGCTACAACGA CCATAGGG 3382 7573 GGAUAUUG G CUAUUUUU 1681 AAAACTAG GGCTAGCTACAACGA ATCCATAG 3382 7577 AUUGGCUA G UUUUGCCU 1682 AGGCAAAA GGCTAGCTACAACGA ATACCATA 3384 7578 CUAGUUUU G CCUUUAUUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA AAAACTAG 3387 7597 UUAAGCAA A UUCAUUCC 1686 GAAATGAA GGCTAGCTACAACGA TAGCCAAT 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA TAATAAA 3387 7601 GCAAAUUC A CUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA TAATAAA 3389 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGAAA GGCTAGCTACAACGA TAAGAATGA 3389 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA TCAGGCTG 3391 7619 GAAUGUCU G CCUAUAUA 1691 TAGGCAGA GGCTAGCTACAACGA ATCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TAGGCAGA GGCTAGCTACAACGA ATCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TAGGCAGA GGCTAGCTACAACGA ATCAGGC 3393 7627 GCCUGAAU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA ATCAGGC 3393 7628 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA ATCAGGC 3394 7629 CUGCCUAU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA ATCAGGC 3394 7621 GCCUGAAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATCAGGA 3394 7625 CUGCUUUU A UAUCUCUC 1696 AGAGAATA GGCTAGCTACAACGA AAAGAGC 3396 7627 GCCUAUAU A UAUCUCUC 1696 AGAGAATA GGCTAGCTACAACGA AAAGAGC 3396 7628 GUUGAACC G UUAAAACA 1699 TATTATAGG GGCTAGCTACAACGA AAAGAGC 3399 7629 UUGAACC G UUAAAACA 1699 TTTTATA GGCTAGCTACAACGA ACAAAGAG 3399 7659 UUGAACCC G UUAAAACA 1699 TTTTATA GGCTAGCTACAACGA ACAAAGAG 3399 7657 GUUAAAAC A CCCGUUAA 1699 TTTTTAA GGCTAGCTACAACGA TC	7545	GUGUAGAU A UAUUUGAC	1673	GICATATA
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7555 AUUGACC A UCALUCG 1677 CCATAGGG GGCTAGCTACAACGA GATGGTCA 3379 7563 AUCACCCU A UGGAUAUU 1678 AATATCCA GGCTAGCTACAACGA AGGGTGAT 3380 7567 CCCUAUGG A UAUUGGCU 1679 AGCCAATA GGCTAGCTACAACGA CCATAGGG 3381 7569 CUAUGGAU A UUGGCUAG 1680 CTAGCCAA GGCTAGCTACAACGA ATCCATAG 3382 7573 GGAUAUUG G CUAUGUUU 1681 AAAACTAG GGCTAGCTACAACGA CATATCC 3383 7577 AUUGGCUA G UUUUGCCU 1682 AGGCAAAA GGCTAGCTACAACGA CAATATCC 3383 7582 CUAGUUUU G CCUUUAUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA TATATAAA 3387 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TATATAAA 3387 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA TATATAAA 3389 7601 GCAAAUUC A UUUCAGCC 1689 GGCTGAGAA GGCTAGCTACAACGA TATATAAA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA ATCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA ATCAGGC 3392 7623 GUCUGCCU A UAUAUUCUU 1693 AGAATATA GGCTAGCTACAACGA AGACATC 3393 7623 GUCUGCCU A UAUAUUCUU 1693 AGAATATA GGCTAGCTACAACGA ATCAGGC 3395 7627 GCCUAUAU A UAUUCUCU 1693 AGAATATA GGCTAGCTACAACGA ATAGAGC 3396 7627 GCCUAUAU A UAUUCUCU 1693 AGAAATATA GGCTAGCTACAACGA ATAGAGC 3396 7627 GCCUAUAU A UAUUCUCU 1693 AGAAATAA GGCTAGCTACAACGA ATAGAGC 3396 7628 CUGCUUU A UAUUCUCU 1694 GCAAGAGA GGCTAGCTACAACGA ATAGAGC 3396 7629 UUGAACC G UUAAAACA 1699 TATATAGG GGCTAGCTACAACGA ATAGAGC 3398 7650 UCCUUUGA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA ATAGAGC 3399 7651 UCCUUUGA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA ATAGAGC 3399 7652 CCGUAAAA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA ATAGAGA 3399 7653 CCCUAAAA A CCCGUUAAA A CAUCCUGU 1697 AAGGAGAA GGCTAGCTACAACGA ATAGAGC 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ATAGAGC 3399 7656 CCGUAAAA A CAUCCUGU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399	7552	UAUAUUUG A CCAUCACC	1675	GG1GK1GG GGC11MG111G1
7563 AUCACCCU A UGGAUAUU 1678 AATATCCA GGCTAGCTACAACGA AGGGTGAT 3380 7567 CCCUAUGG A UAUUGGCU 1679 AGCCAATA GGCTAGCTACAACGA CCATAGGG 3381 7569 CUAUGGAU A UUGGCUAG 1680 CTAGCCAA GGCTAGCTACAACGA ATCCATAG 3382 7573 GGAUAUUG G CUAGUUUU 1681 AAAACTAG GGCTAGCTACAACGA CARTATCC 3383 7577 AUUGGCUA G UUUUGCCU 1682 AGGCAAAA GGCTAGCTACAACGA TAGCCAAT 3384 7582 CUAGUUUU G CCUUUAUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA AAAACTAG 3387 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCAACGA TTGCTTAA 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATCAGGCTG 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA ATCAGGCTG 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGA 3394 7625 CUGCCUAU A UAUAUUCU 1693 AGAGAATA GGCTAGCTACAACGA ATCAGGC 3394 7626 CUGCCUAU A UAUAUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3396 7634 UAUUCUCU G CUCUUUGU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3396 7643 CUCUUUGU A UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA ATAGAGA 3397 7644 UAUUCUCU G CUCUUUGU 1697 AAGAGAAA GGCTAGCTACAACGA AAAGAGG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTACAGGG GGCTAGCTACAACGA AAAGAGA 3398 7667 GCCUAUAAA A CCCGUUAA 1698 TTACAGGG GGCTAGCTACAACGA AAAGAGA 3399 7659 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA AAAGAGA 3399 7659 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA ACAAAGAG 3399 7657 GCCUAUAAA A CCCGUUAA 1698 TTACAGGG GGCTAGCTACAACGA ACAAAGAG 3490 7659 UUGAAACC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA ACAAAGAG 3400 7659 UUGAAACC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAAACC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA TTTAACGG 3400	7555	AUUUGACC A UCACCCUA	1676	INGGGIGN COCINOCITATION: TOTAL
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7567 CCCGAUGG A UAUGGCUA 1690 CTAGCCAA GGCTAGCTACAACGA ATCCATAG 3382 7573 GGAUAUUG G CUAGUUUU 1681 AAAACTAG GGCTAGCTACAACGA CAATATCC 3383 7577 AUUGGCUA G UUUUGCCU 1682 AGGCAAAA GGCTAGCTACAACGA TAGCCAAT 3384 7582 CUAGUUUU G CCUUUAUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA AAAGGCAA 3386 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTAATAAA 3387 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA TTGCTTAA 3388 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGACATTC 3393 7625 CUGCCUAU A UAUAUUCU 1693 AGAGAATA GGCTAGCTACAACGA ATCAGGC 3395 7627 GCCUAUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1693 AGAGAATA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1693 AGAGAATA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 7643 CUCUUUGA A UCUUCUCU 1697 AAGGAGAA GGCTAGCTACAACGA AGAGAATA 3397 7644 UAUUCUCU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7656 CCGUAAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TCAAACGA 3400 7667 GUAAAACA AUCCUGUG 1700 ACAGGATG GGCTAGCTACAACGA GTTTAACG 3400 7667 GUAAAACA AUCCUGUG 1700 ACAGGATG GGCTAGCTACAACGA GTTTAAACG 3400	7563	AUCACCCU A UGGAUAUU	1678	MAINICCA GGCINGCINGCINGCINGCINGCINGCINGCINGCINGCI
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7577 AUUGCCUA G UUUUGCCU 1682 AGGCAAAA GGCTAGCTACAACGA TAGCCAAT 3384 7582 CUAGUUUU G CCUUUAUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA AAAGCCAA 3387 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA TTGCTTAA 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TGAAATGA 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA TCAGGCTG 3391 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA ATTCAGGC 3392 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAATATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATAGGCAG 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA ATATAGGC 3396 7640 UAUUCUCU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGAG 3396 7641 UGCUCUUU G UAUUCUCU 1697 AAGAGAGA GGCTAGCTACAACGA AAAGAGAG 3397 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA AAAGAGAG 3399 7657 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA ACAAAGAG 3399 7667 GUUAAAAC A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7667 GUUAAAAC A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3400 7667 GUUAAAAC A CCCGUUAA 1699 TCTTTTAA GGCTAGCTACAACGA ACAAAGAG 3400	7569	CUAUGGAU A UUGGCUAG	1680	CINGCOM GOCIMOONICE
7577 ADUGACUA G UUUAUUU 1683 AATAAAGG GGCTAGCTACAACGA AAAACTAG 3385 7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAACTAG 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA ATAATAAA 3387 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAA GGCTAGCTACAACGA TTGCTTAA 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TGAAATGA 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA TCAGGCTG 3391 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA ATATAGGC 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 7642 UAUUCUCU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA AAAGAGAG 3399 7657 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA ACAAAGAG 3399 7658 CCGUUAAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TCAAAGGA 3400 7667 GUUAAAAC A UCCUGUGG 1701 ACAGGATG GGCTAGCTACAACGA TCAAAGGA 3400 7667 GUUAAAAC A UCCUGUGG 1701 ACAGGATG GGCTAGCTACAACGA TCAAACGA 3401 7667 GUUAAAAC A UCCUGUGG 1701 ACAGGATG GGCTAGCTACAACGA TCAAACGA 3401	7573	GGAUAUUG G CUAGUUUU	1681	AMMICINO COCINOCITA
7588 UUGCCUUU A UUAAGCAA 1684 TTGCTTAA GGCTAGCTACAACGA AAAGGCAA 3386 7593 UUUAUUAA G CAAAUUCA 1685 TGAATTTG GGCTAGCTACAACGA TTAATAAA 3387 7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA GAATTTGC 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA ATTCAGGC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3396 7627 GCCUAUAU A UUCUCUCC 1694 GCAGAGAA GGCTAGCTACAACGA ATAGGCAG 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA ATATAGGC 3396 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 7642 UGCUCUUU A UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7657 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7667 GUUAAAACA CUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402 7667 GUUAAAACA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3403	7577	AUUGGCUA G UUUUGCCU	1682	AUGCARD: COCINGEING
TOURICCOUR A CHARLEM 1685 TGAATTG GGCTAGCTACAACGA TTAATAAA 3387 T597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 T601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA GAATTTGC 3389 T607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 T613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 T615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 T619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 T623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 T625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 T627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATAGGCA 3396 T634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 T641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3397 T641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAGA 3398 T643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3398 T643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3398 T655 UCCUUUGA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA ACAAAGAG 3399 T655 UCCUUUGA A CCCGUUAA 1698 TTAACGG GGCTAGCTACAACGA ACAAAGAG 3400 T659 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA TCAAAGAG 3401 T665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402 T667 GUUAAAACA UCCUGUG 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402	7582	CUAGUUUU G CCUUUAUU	1683	Williamon occinioning
7597 UUAAGCAA A UUCAUUUC 1686 GAAATGAA GGCTAGCTACAACGA TTGCTTAA 3388 7601 GCAAAUUC A UUUCAGCC 1687 GGCTGAAA GGCTAGCTACAACGA GAATTTGC 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3398 7643 CUCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7667 GUUAAAACA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402 7667 GUUAAAACA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3403	7588	UUGCCUUU A UUAAGCAA	1684	TIGGTIAN GGGZIGGTIG
7597 UUAAGCAR A UUUCAGCC 1687 GGCTGARA GGCTAGCTACAACGA GAATTGC 3389 7607 UCAUUUCA G CCUGAAUG 1688 CATTCAGG GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA TCAAAGGA 3401 7667 GUUAAAAC A UCCUGUG 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7593	UUUAUUAA G CAAAUUCA	1685	IGAITIC COCINCIPAL
7601 GCAAAUUC A UUUCAGCC 1687 GGCTAGCTACAACGA TGAAATGA 3390 7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA ATATAGGC 3396 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAACA 3397 7642 CUCUUUGA A UCUCCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3398 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA TCAAAGGA 3401 7667 GUUAAAACA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAACA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402	7597	UUAAGCAA A UUCAUUUC	1686	Granton Cocinocan
7613 CAGCCUGA A UGUCUGCC 1689 GGCAGACA GGCTAGCTACAACGA TCAGGCTG 3391 7615 GCCUGAAU G UCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA ACAAAGAG 3400 7659 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC	7601	GCAAAUUC A UUUCAGCC	1687	GGC1GrP3. GGC1::GG1::G
7613 CAGCCUGA A UGUCUGCCUA 1690 TAGGCAGA GGCTAGCTACAACGA ATTCAGGC 3392 7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AGAGAATA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TCTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7607	UCAUUUCA G CCUGAAUG	1688	CATTCAGG GGCTAGGTTGTT
7619 GAAUGUCU G CCUAUAUA 1691 TATATAGG GGCTAGCTACAACGA AGACATTC 3393 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA AGGCAGAC 3394 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7669 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7667 GUUAAAAC A UCCUGUG 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402	7613	CAGCCUGA A UGUCUGCC	1689	GGCAGACA GGGZAGG
GAAUGUCU G CCUAUADA 1691 IATATAGG GCTAGCTACAACGA AGGCAGAC 3394 7623 GUCUGCCU A UAUAUUCU 1692 AGAATATA GGCTAGCTACAACGA ATAGGCAG 3395 7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3396 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAAC 3403	7615	GCCUGAAU G UCUGCCUA	1690	TAGGERAT GUELLOUIS
7625 CUGCCUAU A UAUUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATAGGCAG 3395 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7669 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA GTTTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTACAACGA GTTTTAAC 3403	7619	GAAUGUCU G CCUAUAUA	1691	TATATAGG GOCTAGGTTGTT
7625 CUGCCUAU A UACUCUCU 1693 AGAGAATA GGCTAGCTACAACGA ATATAGGC 3396 7627 GCCUAUAU A UUCUCUGC 1694 GCAGAGAA GGCTAGCTACAACGA ATATAGGC 3396 7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7623	GUCUGCCU A UAUAUUCU	1692	AGAATATA OGGTAGGTAGGT
7634 UAUUCUCU G CUCUUUGU 1695 ACAAAGAG GGCTAGCTACAACGA AGAGAATA 3397 7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7625	CUGCCUAU A UAUUCUCU	1693	AGAGMIN COCINGOINGING
7641 UGCUCUUU G UAUUCUCC 1696 GGAGAATA GGCTAGCTACAACGA AAAGAGCA 3398 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7627	GCCUAUAU A UUCUCUGC	1694	GCHGHGHAN GGC271GC
7641 UGCUCUUU G UAOUCUCC 1698 GGAGAATA CGCTAGCTACAACGA ACAAAGAG 3399 7643 CUCUUUGU A UUCUCCUU 1697 AAGGAGAA GGCTAGCTACAACGA ACAAAGAG 3399 7655 UCCUUUGA A CCCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7634	UAUUCUCU G CUCUUUGU	1695	
7643 CUCUUUGA A CUCGUUAA 1698 TTAACGGG GGCTAGCTACAACGA TCAAAGGA 3400 7659 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7641	UGCUCUUU G UAUUCUCC	1696	GGAGANIA GGCINGCON
7655 UUGAACCC G UUAAAACA 1699 TGTTTTAA GGCTAGCTACAACGA GGGTTCAA 3401 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7643	CUCUUUGU A UUCUCCUU	1697	MAGGAGAA GGC1AGC1AGC1AGC1AGC1AGC1AGC1AGC1AGC1AGC1
7659 UUGAACCC G UUAAAACA 1699 IGIIIIAA GGCIAGCTACAACGA TTTAACGG 3402 7665 CCGUUAAA A CAUCCUGU 1700 ACAGGATG GGCTAGCTACAACGA TTTAACGG 3402 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7655	UCCUUUGA A CCCGUUAA	1698	TIAACGGG CGCIAGG
7665 CCGOUAAA A CAUCCUGU 1700 ACAGGAIG GCCTAGCTACAACGA GTTTTAAC 3403 7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGCTACAACGA GTTTTAAC 3403	7659	UUGAACCC G UUAAAACA	1699	IGITIAN GGCINGOLIGITAL
7667 GUUAAAAC A UCCUGUGG 1701 CCACAGGA GGCTAGGAACGA AGGATGTT 3404	7665	CCGUUAAA A CAUCCUGU	1700	ACAGGAIG GGCIAGGIIGE
7672 AACAUCCU G UGGCACUC 1702 GAGTGCCA GGCTAGCTACAACGA AGGATGTT 3404	7667	GUUAAAAC A UCCUGUGG	1701	CCACAGGA GGCIAGGIICE
	7672	AACAUCCU G UGGCACUC	1702	GAGTGCCA GGCTAGCTACAACGA AGGATGTT 3404

Input Sequence = HSFLT. Cut Site = R/Y

Arm Length = 8. Core Sequence = GGCTAGCTACAACGA

HSFLT (Human flt mRNA for receptor-related tyrosine kinase.; Acc# X51602; 7680 bp)

Table VI: Human KDR DNAzyme and Substrate sequence

Pos	Substrate	Seq ID	DNAzyme	Seq IID
TO2	Smart are	_	<i>2021</i> ,223	No
		No	TCCCGGGG GGCTAGCTACAACGA CCCGGGAC	4691
14	GUCCCGGG A CCCCGGGA	3405	ACTGACCG GGCTAGCTACAACGA TCTCCCGG	4692
25	CCGGGAGA G CGGUCAGU	3406	CACACTGA GGCTAGCTACAACGA CGCTCTCC	4693
28	GGAGAGCG G UCAGUGUG	3407	ACCACACA GGCTAGCTACAACGA TGACCGCT	4694
32	AGCGGUCA G UGUGUGGU	3408	CGACCACA GGCTAGCTACAACGA ACTGACCG	4695
34	CGGUCAGU G UGUGGUCG		AGCGACCA GGCTAGCTACAACGA ACACTGAC	4696
36	GUCAGUGU G UGGUCGCU		CGCAGCGA GGCTAGCTACAACGA CACACACT	4697
39	AGUGUGUG G UCGCUGCG		AAACGCAG GGCTAGCTACAACGA GACCACAC	4698
42	GUGUGGUC G CUGCGUUU		AGGAAACG GGCTAGCTACAACGA AGCGACCA	
45	UGGUCGCU G CGUUUCCU		AGGGAAACG GGCTAGCTACAACGA GCAGCGAC	
47	GUCGCUGC G UUUCCUCU		GGCGCAGG GGCTAGCTACAACGA AGAGGAAA	
56	uuuccucu g ccugcgcc			
60	CUCUGCCU G CGCCGGGC		GCCCGGCG GGCTAGCTACAACGA AGGCAGAG	
62	CUGCCUGC G CCGGGCAU		ATGCCCGG GGCTAGCTACAACGA GCAGGCAG	
67	UGCGCCGG G CAUCACUU		AAGTGATG GGCTAGCTACAACGA CCGGCGCA	
69	CGCCGGGC A UCACUUGC		GCAAGTGA GGCTAGCTACAACGA GCCCGGCG	
72	CGGGCAUC A CUUGCGCG		CGCGCAAG GGCTAGCTACAACGA GATGCCCG	
76	AUCACUU G CGCGCCGC		GCGGCGCG GGCTAGCTACAACGA AAGTGATG	
78	UCACUUGC G CGCCGCAC		CTGCGGCG GGCTAGCTACAACGA GCAAGTGA	
80	ACUUGCGC G CCGCAGA		TTCTGCGG GGCTAGCTACAACGA GCGCAAGT	
83	UGCGCGCC G CAGAAAGU	-}	ACTITCIG GGCTAGCTACAACGA GGCGCGCA	
90	CGCAGAAA G UCCGUCUC		CAGACGGA GGCTAGCTACAACGA TTTCTGCC	
94	GAAAGUCC G UCUGGCA		CTGCCAGA GGCTAGCTACAACGA GGACTTTC	
99	UCCGUCUG G CAGCCUG	3 3427	CCAGGCTG GGCTAGCTACAACGA CAGACGG	
102	GUCUGGCA G CCUGGAU	A 3428	TATCCAGG GGCTAGCTACAACGA TGCCAGAG	
108	CAGCCUGG A UAUCCUC	U 3429	AGAGGATA GGCTAGCTACAACGA CCAGGCT	
110	GCCUGGAU A UCCUCUC	C 3430	GGAGAGGA GGCTAGCTACAACGA ATCCAGG	
120	CCUCUCCU A CCGGCAC	C 3431	GGTGCCGG GGCTAGCTACAACGA AGGAGAG	
124	UCCUACCG G CACCCGC	A 3432	TGCGGGTG GGCTAGCTACAACGA CGGTAGG	
126	CUACCGGC A CCCGCAG	A 3433	TCTGCGGG GGCTAGCTACAACGA GCCGGTA	
130	CGGCACCC G CAGACGC	C 3434	GGCGTCTG GGCTAGCTACAACGA GGGTGCC	
134	ACCCGCAG A CGCCCCU	G 3435	CAGGGGCG GGCTAGCTACAACGA CTGCGGG	
130	CCGCAGAC G CCCCUGC	A 3436	TGCAGGGG GGCTAGCTACAACGA GTCTGCG	
14:	2 ACGCCCCU G CAGCCGC	C 3437	GGCGGCTG GGCTAGCTACAACGA AGGGGCG	
14	5 CCCCUGCA G CCGCCGG	IU 3438	ACCGCCG GGCTAGCTACAACGA TGCAGGG	
14	8 CUGCAGCC G CCGGUCG	G 3439	CCGACCGG GGCTAGCTACAACGA GGCTGCA	
15	2 AGCCGCCG G UCGGCGC	C 3440	GGCGCCGA GGCTAGCTACAACGA CGGCGGC	
15	6 GCCGGUCG G CGCCCGG	G 3441	CCCGGGCG GGCTAGCTACAACGA CGACCGG	
15	8 CGGUCGGC G CCCGGGC	U 3442	AGCCCGGG GGCTAGCTACAACGA GCCGACC	
16	4 GCGCCCGG G CUCCCU	G 3443	CTAGGGAG GGCTAGCTACAACGA CCGGGCC	
	2 GCUCCCUA G CCCUGUC		GCACAGGG GGCTAGCTACAACGA TAGGGAC	
17			TGAGCGCA GGCTAGCTACAACGA AGGGCT	AG 4731
17			GTTGAGCG GGCTAGCTACAACGA ACAGGG	
18			CAGTTGAG GGCTAGCTACAACGA GCACAG	3G 4733
18			CAGGACAG GGCTAGCTACAACGA TGAGCG	CA 4734
18			GCGCAGGA GGCTAGCTACAACGA AGTTGA	3C 4735
19			CCGCAGCG GGCTAGCTACAACGA AGGACA	GT 4736
19			CCCCGCAG GGCTAGCTACAACGA GCAGGA	
19			GCACCCCG GGCTAGCTACAACGA AGCGCA	
1 73	4 GCUGCGGG G UGCCGC		TOTAL TOTAL CONTRACTOR OF COCCOS	

206 UGCOGGOU G CCGCGAGU 3454 ACTGCGGG GGCTACCATCAAACGA GGCACCCC 4740 209 GGGGGGC G GAGUUCC 3455 AGGTGGGA G GUCCACCU 3455 AGGTGGAA GGCTACAAACGA GGCACCCC 4742 218 GGGGGGCGA G UUCCACCU 3456 AGGTGGAA GGCTACAAACGA GGAGGTGC 4742 218 GAGGUUCCA C CCUCCUCU 3458 AGGGGGG GGCTAGCTACAACGA GGAGGTG 4745 224 CUACCUCC G CGCCUCCU 3458 AGGGCTTG GGCTACCAACGA GGGGTAGCTACAACGA GGGTAGCTACAACGA CGTTCTACA 4745 240 DUCUCUGA A CAGGGGCU 3460 AGGGCTTG GGCTACCAACGA CGTTCTCA 4745 244 CUIRGACG G CUCCGGGA 3461 TCCCACGG GGCTAGCTACAACGA CGTTCTC 4748 259 GAGAAAGA A CCGGCCCC 3463 GGAGACCG G CUCCCAGGG 3462 TCTCCCAG GGCTAGCTACAACGA CGTTCTC 4749 271 GCUCCCGA G UUCUGGGC 3465 CCCCAGAA GGCTAGCTACAACGA CGTCTCT 4751 272 GCUCCCGA G UUCUGGGC 3466 CCCAGAAA GGCTAGCTACAACGA CCCAGAA 4752 280 UUCUGGG C CCUCGGGGU 3469 ACCTCGAGA GGCTAGCTACAACGA CCCAGAAA 4752 <th></th> <th></th> <th></th> <th>128</th> <th></th>				128	
209 GGGGUGCC G CGAGUUCC 3455 GGARACTCG GCTACAACGA GGCACCCC 4741 213 UGCCGCCA G UUCCACCU 3455 AGSTGGAA GGCTAGCTACAACGA TCGCGGCA 4743 218 CGAGUUCC A CCUCCUCU 3455 AGSTGGAA GGCTAGCTACAACGA GGAACTCG 4743 224 ACCUCCUC G CCUCCUU 3459 AMAGAGG GGCTAGCTACAACGA GGAGGAGT 4745 226 ACCUCCUC G CCUCCUU 3459 AMAGAGG GGCTAGCTACAACGA CGAGGAGT 4745 240 UUCUCUAG A CAGGCCU 3469 AGGTGGCG GGCTAGCTACAACGA CGTGTAGTACATCA CGACGA CTGTTAG 241 CURARCAG G CUGGGAA 3461 TCCCCAGG GGCTAGCTACAACGA CTGTTCTC 4749 246 AGRANGGC G CUGGGAA 3462 TCTCCCAG GGCTAGCTACAACGA CGGGTTCT 4748 263 AAGAACG G CUCCCAGA 3464 CTCGGGAG GGCTAGCTACAACGA CGGGGAC 4751 270 GGUCCCAG G UUCUGGC 3466 GCCAAAAA GGCTAGCTACAACGA CGGGACC 4751 280 UUCUGGG G CAUUUCGC 3467 GGGCGAAA GGCTAGCTACAACGA CGAGACC 4752 280 UUCUGGGC A UUUCGCC 3467 AGGGCGAGG GGCTAGCTACAACGA CAGAAACGA 4753 281 GGCAUUUC G CCCGGGCCU 3468 ACCTCGAG GGCTAGCTACAACGA CAGAAACGA 4752 287 GGCCCCAGA GGCTAGCTACAACGA CACCAGAAA 3471 ACTCCTCAG GGCTAGCTACAACGA ACCTCGAAC	206	UGCGGGGU G CCGCGAGU	3454	ACTCGCGG GGCTAGCTACAACGA ACCCCGCA 4	740
213 UGCGGGGA G UUCCACCU 3455 AGSTGGGA GGCTAGCTACAAGGA TGGGGGCA 4742 218 GGAGUUCC A CCUCCCCU 3455 AGGGGGGG GGCTAGCTACAACGA GGAACTCG 4743 224 CCACCUCC G GGCCUCCU 3458 AGGAGGG GGCTAGCTACAACGA GGAGGGTG 4745 224 CCACCUCG G GCCUCCUU 3458 AGGAGGG GGCTAGCTACAACGA GGAGGGGT 4745 240 TULUUCUGA A CAGGGCCU 3460 AGGGCCTG GGCTAGCTACAACGA CTAGAGGA 4746 241 CUAGAGG G GCUGGGGA 3461 TCCCCAGG GGCTAGCTACAACGA CCTAGAGGA 7474 242 GAGACAGG G CUCCCGAGG 3463 GGAGCCGG GGCTAGCTACAACGA CCGTTCTT 4748 253 AGGAACAG G CUCCCGAGG 3464 CTCGGGGG GGCTAGCTACAACGA CCGTTCT 4752 274 AGUUCGGG G CUCCCGAGG 3465 CCCCAGAA GGCTAGCTACAACGA CCGACCT 4752 275 AGUUCGGG C AUUUCGCC 3466 CCCAGAAA GGCTAGCTACAACGA CCGACACT 4752 286 UUCGGGC G CUCCAGGGU 3468 AGACCGGG GGCTAGCTACAACGA CCGAAACT 4752 287 GGCCUCGAG G UCCAAGGAU 3479 ACCTCGAG GGCTAGCTACAACGA CAGAAAACAC 4754 289 UUCGCCCG G CUCGAGGU 3469 ACCTCGAG GGCTAGCTACAACGA CACGACCC 4758 304 GCGCAGAGA UGCAACAA 3471 ACCTCGAG GGCTAGCTACAACGA CACCACCA 4756 305 UUCGACCG G CAGGGGC 3479 ACCTCGAG GGCTAGCTACAACGA A	┝╾╼┵		3455	GGAACTCG GGCTAGCTACAACGA GGCACCCC 4	741
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346 GGCUCUGC G UGGAGACC 3484 GGCCTCCA GGCTAGCTACAACGA GCAGAGCC 4770 352 GCGUGGAG A CCCGGGCC 3485 GGCCCGGG GGCTAGCTACAACGA CTCCACGC 4771 358 AGACCCGG G CCGCCUCU 3486 AGAGGCGG GGCTAGCTACAACGA CCGGGTCT 4772 361 CCCGGGCC G CCUCUGUG 3487 CACAGAGG GGCTAGCTACAACGA GGCCCGGG 4773 367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA AGAGGCGG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUCU 3491 GAGAAACA GGCTAGCTACAACGA AAACCCAC 4776 382 UGCCUAGU G UUUUCUCU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA ACTAGGCA 4778 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA AGATCAAG 4780 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGGG GGCTAGCTACAACGA AGATCAAG 4780 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTTGTA GGCTAGCTACAACGA ATGCTAGA 4784 419 ACAAAAAGA A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTAGA 4784 419 ACAAAAAGA A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTAGA 4784 419 ACAAAAAGA A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAGA A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAGA A UACUUACAA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTT 4786 421 AAAAAGAC A UACUUACAA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3500 TGTAAGTA GGCTAGCTACAACGA ATGTCTTT 4787 426 ACAUACUU A CAAUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGTCTTT 4786 427 ACAUACUU A CAAUUACA 3501 TGTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA A UUACAACU 3501 TGTAATTG GGCTAGCTACAACGA CTTAATTG 4780 436 CAAUUAAG G CUAAUACA 3501 TGTAATTG GGCTAGCTACAACGA CTTAATTG 4780 436 CAAUUAAG G CUAAUACA 3501 TGTAATTG GGCTAGCTACAACGA CTTAATTG 4790	339	GCCCUGUG G CUCUGCGU	3482	ACGCAGAG GGCTAGCTACAACGA CACAGGGC	4768
345 GCCUCGC G GCGGCC 3485 GGCCCGGG GGCTAGCTACAACGA CTCCACGC 4771 358 AGACCCGG G CCGCCUCU 3486 AGAGGCGG GGCTAGCTACAACGA CCGGGTCT 4772 361 CCCGGGCC G CCUCUGUG 3487 CACAGAGG GGCTAGCTACAACGA GGCCCGGG 4773 367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA AGAGGCGG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUCU 3491 GAGAAACA GGCTAGCTACAACGA AAACCCAC 4776 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA ACTAGGCA 4778 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA AGATCAAG 4780 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA AGATCAAG 4781 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3498 TCTTTTGTA GGCTAGCTACAACGA ATGCTGAG 4784 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAGA CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAGA CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTTTTT 4786 421 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 424 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGTCTTT 4786 425 ACAUACUU A CAAUAAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 426 ACAUACUU A CAAUAACA 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 427 ACAUACUU A CAAUAACA 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA UACAACUC 3503 AGCCTTAA GGCTAGCTACAACGA ATGTCTTT 4786 431 UACUUACA UACAACUC 3503 AGCCTTAA GGCTAGCTACAACGA CTTAATTG 4790 432 ACAUACUU A CAAUACAC 3503 AGCCTTAA GGCTAGCTACAACGA CTTAATTG 4790 433 UACUUACA A UACAACUC 3505 GAGTTGTA GGCTACCAACGA CTTAATTG 4790 4440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTACCAACGA CTTAATTG 4790	344	GUGGCUCU G CGUGGAGA	3483	TCTCCACG GGCTAGCTACAACGA AGAGCCAC	4769
358 AGACCCGG G CCGCCUCU 3486 AGAGGCGG GGCTAGCTACAACGA CCGGGTCT 4772 361 CCCGGGCC G CCUCUGUG 3487 CACAGAGG GGCTAGCTACAACGA GGCCCGGG 4773 367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA CACAGAGG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA CACAGAGG 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACACCAC 4776 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA ACAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA CACAGAGAA 4779 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA AGATCAAG 4780 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA TGAGCCTG 4783 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAGA A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4786 421 AAAAAGA A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTATT 4786 422 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 424 ACAUACUU A CAAUUAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA ATGTCTTT 4788 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA TGTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TTAATTG 4790	346	GGCUCUGC G UGGAGACO	3484	GGTCTCCA GGCTAGCTACAACGA GCAGAGCC	4770
358 AGACCCGG G CCGCCUCU 3486 AGAGGCGG GGCTAGCTACAACGA CCGGGTCT 4772 361 CCCGGGCC G CCUCUGUG 3487 CACAGAGG GGCTAGCTACAACGA GGCCCGGG 4773 367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA CCACAGAG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA ACTAGGCA 4778 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA CAAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA AGATCAAG 4780 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA CTGGGCAG 4781 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA CTGGGCAG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA ATGCTGAG 4784 411 CUCAGCAU A CAAAAAAA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4786 421 AAAAAGA A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA CTTTTTTT 4785 421 AAAAAGA A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 424 ACAUACUU A CAAUUAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 425 ACAUACUU A CAAUUAAG 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA CTTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA TGTAAGTA 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTA GGCTAGCTACAACGA TTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TTAATTG 4790	352	GCGUGGAG A CCCGGGCC	3485	GGCCCGGG GGCTAGCTACAACGA CTCCACGC	4771
361 CCCGGGCC G CCUCUGUS 3487 CACAGAGG GGCTAGCTACAACGA GGCCCGGG 4773 367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA CCACAGAG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA CAAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA AGATCAAG 4780 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA CTGGGCAG 4781 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA GTGAGCCT 4783 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA GTCTTTTTT 4786 421 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4787 426 ACAUACUU A CAAUUAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA TGTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA TGTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA TGTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA TGTAATTG 4780 437 UACGCUAA A UACAACUC 3505 GAGTTGTA GGCCTAGCTACAACGA TAGCCTTA 4781				AGAGGCGG GGCTAGCTACAACGA CCGGGTCT	4772
367 CCGCCUCU G UGGGUUUG 3488 CAAACCCA GGCTAGCTACAACGA AGAGGCGG 4774 371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA CCACAGAG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA CAAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA CAAGAGAA 4779 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA CTGGGCAG 4781 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA GTGAGCC 4783 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA CTTTTTGT 4785 421 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA GTCTTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4787 427 ACAUACUU A CAAUUAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4786 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA TGTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA CTTAATTG 4780 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA TGTAATTG 4780 430 UACGCUAA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA CTTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TAGCCTTA 4781				CACAGAGG GGCTAGCTACAACGA GGCCCGGG	4773
371 CUCUGUGG G UUUGCCUA 3489 TAGGCAAA GGCTAGCTACAACGA CCACAGAG 4775 375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA CAAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA CAGAGAAA 4779 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA CTGGGCAG 4780 403 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA TGAGCCTG 4783 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA CTTTTTGT 4785 421 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA ATGCTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4787 426 ACAUACUU A CAAUAAG 3502 CTTAATTG GGCTAGCTACAACGA ATGTCTTT 4788 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA TGTAAGTA 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA CTTAATTG 4789 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA CTTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TAGCCTTA 4791				CAAACCCA GGCTAGCTACAACGA AGAGGCGG	4774
375 GUGGGUUU G CCUAGUGU 3490 ACACTAGG GGCTAGCTACAACGA AAACCCAC 4776 380 UUUGCCUA G UGUUUCUC 3491 GAGAAACA GGCTAGCTACAACGA TAGGCAAA 4777 382 UGCCUAGU G UUUCUCUU 3492 AAGAGAAA GGCTAGCTACAACGA ACTAGGCA 4778 392 UUCUCUUG A UCUGCCCA 3493 TGGGCAGA GGCTAGCTACAACGA CAAGAGAA 4779 396 CUUGAUCU G CCCAGGCU 3494 AGCCTGGG GGCTAGCTACAACGA AGATCAAG 4780 402 CUGCCCAG G CUCAGCAU 3495 ATGCTGAG GGCTAGCTACAACGA CTGGGCAG 4781 407 CAGGCUCA G CAUACAAA 3496 TTTGTATG GGCTAGCTACAACGA TGAGCCTG 4782 409 GGCUCAGC A UACAAAAA 3497 TTTTTGTA GGCTAGCTACAACGA GCTGAGCC 4783 411 CUCAGCAU A CAAAAAGA 3498 TCTTTTTG GGCTAGCTACAACGA ATGCTGAG 4784 419 ACAAAAAG A CAUACUUA 3499 TAAGTATG GGCTAGCTACAACGA CTTTTTGT 4785 421 AAAAAGAC A UACUUACA 3500 TGTAAGTA GGCTAGCTACAACGA GTCTTTTT 4786 423 AAAGACAU A CUUACAAU 3501 ATTGTAAG GGCTAGCTACAACGA ATGTCTTT 4787 427 ACAUACUU A CAAUUAAG 3502 CTTAATTG GGCTAGCTACAACGA AAGTATGT 4788 430 UACUUACA A UUAAGGCU 3503 AGCCTTAA GGCTAGCTACAACGA CTTAATTG 4790 436 CAAUUAAG G CUAAUACA 3504 TGTATTAG GGCTAGCTACAACGA CTTAATTG 4790 440 UAAGGCUA A UACAACUC 3505 GAGTTGTA GGCTAGCTACAACGA TAGCCTTA 4791					4775
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445	CUAAUACA A CUCUUCAA	3507	TTGAAGAG GGCTAGCTACAACGA TGTATTAG 4793
454	CUCUUCAA A UUACUUGC	3508	GCAAGTAA GGCTAGCTACAACGA TTGAAGAG 4794
		3509	CCTGCAAG GGCTAGCTACAACGA AATTTGAA 4795
		3510	GTCCCCTG GGCTAGCTACAACGA AAGTAATT 4796
	UGCAGGGG A CAGAGGGA	3511	TCCCTCTG GGCTAGCTACAACGA CCCCTGCA 4797
	ACAGAGGG A CUUGGACU	3512	AGTCCAAG GGCTAGCTACAACGA CCCTCTGT 4798
	GGACUUGG A CUGGCUUU	3513	AAAGCCAG GGCTAGCTACAACGA CCAAGTCC 4799
	UUGGACUG G CUUUGGCC	3514	GGCCAAAG GGCTAGCTACAACGA CAGTCCAA 4800
	UGGCUUUG G CCCAAUAA	3515	TTATTGGG GGCTAGCTACAACGA CAAAGCCA 4801
	UUGGCCCA A UAAUCAGA	3516	TCTGATTA GGCTAGCTACAACGA TGGGCCAA 4802
	GCCCAAUA A UCAGAGUG	3517	CACTCTGA GGCTAGCTACAACGA TATTGGGC 4803
		3518	CACTGCCA GGCTAGCTACAACGA TCTGATTA 4804
	UAAUCAGA G UGGCAGUG		GCTCACTG GGCTAGCTACAACGA CACTCTGA 4805
509	UCAGAGUG G CAGUGAGC	3519	TTTGCTCA GGCTAGCTACAACGA TGCCACTC 4806
512	GAGUGGCA G UGAGCAAA	3520	
516	GGCAGUGA G CAAAGGGU	3521	7.0001110
523	AGCAAAGG G UGGAGGUG	3522	CACCTCCA GGCTAGCTACAACGA CCTTTGCT 4808
529	GGGUGGAG G UGACUGAG	3523	CTCAGTCA GGCTAGCTACAACGA CTCCACCC 4809
532	UGGAGGUG A CUGAGUGC	3524	GCACTCAG GGCTAGCTACAACGA CACCTCCA 4810
537	GUGACUGA G UGCAGCGA	3525	TCGCTGCA GGCTAGCTACAACGA TCAGTCAC 4811
539	GACUGAGU G CAGCGAUG		CATCGCTG GGCTAGCTACAACGA ACTCAGTC 4812
542	UGAGUGCA G CGAUGGCC	3527	GGCCATCG GGCTAGCTACAACGA TGCACTCA 4813
545	GUGCAGCG A UGGCCUCU	3528	AGAGGCCA GGCTAGCTACAACGA CGCTGCAC 4814
548	CAGCGAUG G CCUCUUCU	3529	AGAAGAGG GGCTAGCTACAACGA CATCGCTG 4815
557	CCUCUUCU G UAAGACAC	3530	GTGTCTTA GGCTAGCTACAACGA AGAAGAGG 4816
562	UCUGUAAG A CACUCACA	3531	TGTGAGTG GGCTAGCTACAACGA CTTACAGA 4817
564	UGUAAGAC A CUCACAAU	3532	ATTGTGAG GGCTAGCTACAACGA GTCTTACA 4818
568	AGACACUC A CAAUUCCA	3533	TGGAATTG GGCTAGCTACAACGA GAGTGTCT 4819
571	CACUCACA A UUCCAAAA	3534	TTTTGGAA GGCTAGCTACAACGA TGTGAGTG 4820
580	UUCCAAAA G UGAUCGGA	3535	TCCGATCA GGCTAGCTACAACGA TTTTGGAA 4821
583	CAAAAGUG A UCGGAAAU	3536	ATTTCCGA GGCTAGCTACAACGA CACTTTTG 4822
590	GAUCGGAA A UGACACUG	3537	CAGTGTCA GGCTAGCTACAACGA TTCCGATC 4823
593	CGGAAAUG A CACUGGAG	3538	CTCCAGTG GGCTAGCTACAACGA CATTTCCG 4824
595		·	GGCTCCAG GGCTAGCTACAACGA GTCATTTC 4825
601			CTTGTAGG GGCTAGCTACAACGA TCCAGTGT 4826
605		 	AGCACTTG GGCTAGCTACAACGA AGGCTCCA 4827
609		 	TAGAAGCA GGCTAGCTACAACGA TTGTAGGC 4828
611			GGTAGAAG GGCTAGCTACAACGA ACTTGTAG 4829
617		+	TTTCCCGG GGCTAGCTACAACGA AGAAGCAC 4830
—			CAAGTCAG GGCTAGCTACAACGA TTCCCGGT 4831
625			AGGCCAAG GGCTAGCTACAACGA CAGTTTCC 4832
629			GACCGAGG GGCTAGCTACAACGA CAAGTCAG 4833
634			ATAAATGA GGCTAGCTACAACGA CGAGGCCA 4834
640		_	GACATAAA GGCTAGCTACAACGA GACCGAGG 4835
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653			CTTGAACA GGCTAGCTACAACGA AGACATAA 4838
655			ATCTTGAA GGCTAGCTACAACGA ATAGACAT 4839
662	UGUUCAAG A UUACAGA	U 3554	ATCTGTAA GGCTAGCTACAACGA CTTGAACA 4840
665	UCAAGAUU A CAGAUCU	C 3555	GAGATCTG GGCTAGCTACAACGA AATCTTGA 4841
669	GAUUACAG A UCUCCAU	U 3556	AATGGAGA GGCTAGCTACAACGA CTGTAATC 4842
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675	AGAUCUCC A UUUAUUG	C 3557	GCAATAAA GGCTAGCTACAACGA GGAGATCT 4843
679			GCAATAAA GGCTAGCTACAACGA GGAGATCT 4843 AGAAGCAA GGCTAGCTACAACGA AAATGGAG 4844

			130	
688	UUGCUUCU G UUAGUGAC	3560	GTCACTAA GGCTAGCTACAACGA AGAAGCAA 4	846
692	UUCUGUUA G UGACCAAC	3561	GTTGGTCA GGCTAGCTACAACGA TAACAGAA 4	847
695	UGUUAGUG A CCAACAUG	3562	CATGTTGG GGCTAGCTACAACGA CACTAACA 4	848
		3563	ACTCCATG GGCTAGCTACAACGA TGGTCACT 4	849
			CGACTCCA GGCTAGCTACAACGA GTTGGTCA 4	850
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	UGUACAUU A CUGAGAAC	3570		856
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725	UACUGAGA A CAAAAACA		1011110 0001110011111111111111111111111	1858
731	GAACAAAA A CAAAACUG	3572		1859
	AAAACAAA A CUGUGGUG	3573	CACCATO COLLEGE	4860
739	ACAAAACU G UGGUGAUU	3574	Art Cracks	4861
742	AAACUGUG G UGAUUCCA	3575	IGG/MICH GOODMOONING	4862
745	CUGUGGUG A UUCCAUGU	3576	Actional Country of the Country of t	
750	GUGAUUCC A UGUCUCGG	3577	CCGAGACA CCCIACO	4863
752	GAUUCCAU G UCUCGGGU	3578	ACCCOACT. ODD	4864
759	UGUCUCGG G UCCAUUUC	3579	Old Bill Coll. College	4865
763	UCGGGUCC A UUUCAAAU	3580	Alliant Continue	4866
770	CAUUUCAA A UCUCAACG	3581	00:10:0:	4867
776	AAAUCUCA A CGUGUCAC	3582	GIONOTCO COCINCE	4868
778	AUCUCAAC G UGUCACUU	3583	AAGTGACA GGCTAGCTACAACGA GTTGAGAT	4869
780	CUCAACGU G UCACUUUG	3584	CAAAGTGA GGCTAGCTACAACGA ACGTTGAG	4870
783	AACGUGUC A CUUUGUGC	3585	GCACAAAG GGCTAGCTACAACGA GACACGTT	4871
788	GUCACUUU G UGCAAGAU	3586	ATCTTGCA GGCTAGCTACAACGA AAAGTGAC	4872
790	CACUUUGU G CAAGAUAC	3587	GTATCTTG GGCTAGCTACAACGA ACAAAGTG	4873
795	UGUGCAAG A UACCCAGA	3588	TCTGGGTA GGCTAGCTACAACGA CTTGCACA	4874
797	UGCAAGAU A CCCAGAAA	3589	TTTCTGGG GGCTAGCTACAACGA ATCTTGCA	4875
810	GAAAAGAG A UUUGUUCC	3590	GGAACAAA GGCTAGCTACAACGA CTCTTTTC	4876
814			ATCAGGAA GGCTAGCTACAACGA AAATCTCT	4877
821			TGTTACCA GGCTAGCTACAACGA CAGGAACA	4878
824			TTCTGTTA GGCTAGCTACAACGA CATCAGGA	4879
827			AAATTCTG GGCTAGCTACAACGA TACCATCA	4880
832			CCAGGAAA GGCTAGCTACAACGA TCTGTTAC	4881
ļ			TCTTGCTG GGCTAGCTACAACGA CCCAGGAA	4882
842		+	CCTTCTTG GGCTAGCTACAACGA TGTCCCAG	4883
845			TAGTAAAG GGCTAGCTACAACGA CCTTCTTG	4884
854			GGGAATAG GGCTAGCTACAACGA AAAGCCCT	4885
859			GCTGGGAA GGCTAGCTACAACGA AGTAAAGC	4886
862			TCATGTAG GGCTAGCTACAACGA TGGGAATA	4887
869				4888
872			TGATCATG GGCTAGCTACAACGA AGCTGGGA	4889
874	CCAGCUAC A UGAUCAGO		GCTGATCA GGCTAGCTACAACGA GTAGCTGG	
877			ATAGCTGA GGCTAGCTACAACGA CATGTAGC	4890
883			CAGCATAG GGCTAGCTACAACGA TGATCATG	
884			TGCCAGCA GGCTAGCTACAACGA AGCTGATC	
886	UCAGCUAU G CUGGCAUG	3 3607	CATGCCAG GGCTAGCTACAACGA ATAGCTGA	4893
89	CUAUGCUG G CAUGGUCL	3608	AGACCATG GGCTAGCTACAACGA CAGCATAG	4894
89	2 AUGCUGGC A UGGUCUUC	3609	GAAGACCA GGCTAGCTACAACGA GCCAGCAT	4895
89!	5 CUGGCAUG G UCUUCUGU	J 3610	ACAGAAGA GGCTAGCTACAACGA CATGCCAG	4896
90:	2 GGUCUUCU G UGAAGCAA	A 3611	TTGCTTCA GGCTAGCTACAACGA AGAAGACC	4897
90			AATTITTG GGCTAGCTACAACGA TTCACAGA	4898
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913	AAGCAAAA A UUAAUGAU	3613	ATCATTAA GGCTAGCTACAACGA TTTTGCTT 489	99
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		3617	TAGACTGG GGCTAGCTACAACGA AACTTTCA 49	03
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	ACCAGUCU A UUAUGUAC	3619		05
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	UCUAUUAU G UACAUAGU	3621		07
942	UAUUAUGU A CAUAGUUG	3622		800
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955	UAGUUGUC G UUGUAGGG	3626	CCCIACAN OCCINOCINATION	913
958	UUGUCGUU G UAGGGUAU	3627	AIACCCIA GGCIAGGERGE	914
963	GUUGUAGG G UAUAGGAU	3628	MICCIAIN COULINGEING	915
965	UGUAGGGU A UAGGAUUU	3629	WMICCIN COCINOCIAL	916
970	GGUAUAGG A UUUAUGAU	3630	AICAIAAA COCIACCEALCE	917
974	UAGGAUUU A UGAUGUGG	3631	CONCRECA COCINOCINAZIONE	
977	GAUUUAUG A UGUGGUUC	3632	Gracer Cocinetical	918
979	UUUAUGAU G UGGUUCUG	3633	CAGAACCA GGCIAGGIAGI	919
982	AUGAUGUG G UUCUGAGU	3634	ACTUACIES COCCESSION	920
989	GGUUCUGA G UCCGUCUC	3635	GAGACGGA GGCIIIGGGIIGGGI	921
993	CUGAGUCC G UCUCAUGG	3636	CCATGAGA GGCTAGCTAGT	922
998	UCCGUCUC A UGGAAUUG	3637	Chilical documents	923
1003	CUCAUGGA A UUGAACUA	3638	INGII CAN OGCINGOC	1924
1008	GGAAUUGA A CUAUCUGU	3639	ACACATAC COCTACCTACTAC	925
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ļ	1 UGAACUAA A UGUGGGGA			4936
	AACUAAAU G UGGGGAUU			4937
	9 AUGUGGGG A UUGACUUC	+		4938
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	9 UGACUUCA A CUGGGAAU		ATTCCCAG COCTAGETACT	4941
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112	4 ACUUGUAA A CCGAGAC	C 3663	GGTCTCGG GGCTAGCTACAACGA TTACAAGT	4949
L	the same of the sa	- 1	TTTTTAGG GGCTAGCTACAACGA CTCGGTTT	4950
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	765 ATTAGAGA GGCTAGCTACAACGA TTCTCACC	
	766 AGGAGAGA GGCTAGCTACAACGA TAGAGATT 767 GGAATCCA GGCTAGCTACAACGA AGGAGAGA	
		
		
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1683 UGGUAUUG G CAGUUGGA 3792	10071010 000111011111111111111111111111
1686 UAUUGGCA G UUGGAGGA 3793	Total Cooling
1698 GAGGAAGA G UGCGCCAA 3794	11000001 0001110111011
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1798 AAAUUGAA G UUAAUAAA 3814	CONTRACTOR OF THE PARTY OF THE
1802 UGAAGUUA A UAAAAAUC 3815	
1808 UAAUAAAA A UCAAUUUG 3816	5100
1812 AAAAAUCA A UUUGCUCU 3817	
1816 AUCAAUUU G CUCUAAUU 3818	
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1835 AGGAAAAA A CAAAACUG 3820	TOUTHER CONTINUE TO THE PROPERTY OF THE PROPER
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1843 ACAAAACU G UAAGUACC 3822	
1847 AACUGUAA G UACCCUUG 3823	disconi oddinodinasioni
1849 CUGUAAGU A CCCUUGUU 3824	AACAAGGG GGCTAGCTACAACGA ACTTACAG 5110

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	CAAAUGU G UCAGCUUU			5118
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2151 GCCAC	CAU G UUCUCUAA	3896	TTAGAGAA GGCTAGCTACAACGA ATGGTGGC	5182
2159 GUUCU	CUA A UAGCACAA	3897	TTGTGCTA GGCTAGCTACAACGA TAGAGAAC	5183
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2164 CUAAU	AGC A CAAAUGAC	3899	GTCATTTG GGCTAGCTACAACGA GCTATTAG	5185
2168 UAGCA	CAA A UGACAUUU	3900	AAATGTCA GGCTAGCTACAACGA TTGTGCTA	5186
2171 CACAA	AUG A CAUUUUGA	3901	TCAAAATG GGCTAGCTACAACGA CATTTGTG	5187
2173 CAAAU	GAC A UUUUGAU	3902	GATCAAAA GGCTAGCTACAACGA GTCATTTG	5188
2179 ACAUU	UUG A UCAUGGAO	3903	CTCCATGA GGCTAGCTACAACGA CAAAATGT	5189
2182 00000	AUC A UGGAGCUU	3904	AAGCTCCA GGCTAGCTACAACGA GATCAAAA	5190
2187 AUCAU	GGA G CUUAAGA	3905	TTCTTAAG GGCTAGCTACAACGA TCCATGAT	5191
2195 GCUUI	AGA A UGCAUCCI	3906	AGGATGCA GGCTAGCTACAACGA TCTTAAGC	5192
2197 UUAAC	AAU G CAUCCUU	3 3907	CAAGGATG GGCTAGCTACAACGA ATTCTTAA	5193
2199 AAGA/	UGC A UCCUUGC	A 3908	TGCAAGGA GGCTAGCTACAACGA GCATTCTT	5194
2205 GCAU	CUU G CAGGACC	A 3909	TGGTCCTG GGCTAGCTACAACGA AAGGATGC	5195
2210 CUUG	AGG A CCAAGGA	G 3910	CTCCTTGG GGCTAGCTACAACGA CCTGCAAG	5196
2219 CCAA	GAG A CUAUGUC	U 3911	AGACATAG GGCTAGCTACAACGA CTCCTTGG	5197
2222 AGGA(ACU A UGUCUGO	C 3912	GGCAGACA GGCTAGCTACAACGA AGTCTCCT	5198
2224 GAGA	UAU G UCUGCCU	U 3913	AAGGCAGA GGCTAGCTACAACGA ATAGTCTC	5199
	UCU G CCUUGCU	-{	GAGCAAGG GGCTAGCTACAACGA AGACATAG	5200
	CUU G CUCAAGA		GTCTTGAG GGCTAGCTACAACGA AAGGCAGA	5201
	AAG A CAGGAAG		TCTTCCTG GGCTAGCTACAACGA CTTGAGCA	5202
	AAG A CCAAGAA		TTTCTTGG GGCTAGCTACAACGA CTTCCTGT	5203
	AAG A CAUUGCG		ACGCAATG GGCTAGCTACAACGA CTTTTCTT	5204
	GAC A UUGCGUG		CCACGCAA GGCTAGCTACAACGA GTCTTTTC	 -
	CAUU G CGUGGUC		TGAČCACG GGCTAGCTAČAACGA AATGTCTT	
	JUGC G UGGUCAG		CCTGACCA GGCTAGCTACAACGA GCAATGTC	
\ 	GUG G UCAGGCA		CTGCCTGA GGCTAGCTACAACGA CACGCAAT	
			GTGAGCTG GGCTAGCTACAACGA CTGACCAC	
	JCAG G CAGCUCA		ACTGTGAG GGCTAGCTACAACGA CTGACCAC	 -
	GGCA G CUCACAG	 		
	CUC A CAGUCCU		TAGGACTG GGCTAGCTACAACGA GAGCTGCC	
<u> </u>	CACA G UCCUAGA		CTCTAGGA GGCTAGCTACAACGA TGTGAGCT	
	JAGA G CGUGUGG		GCCACACG GGCTAGCTACAACGA TCTAGGAC	
	BAGC G UGUGGCA		GTGCCACA GGCTAGCTACAACGA GCTCTAGG	
	GCGU G UGGCACO		GGGTGCCA GGCTAGCTACAACGA ACGCTCTA	·
2299 AGCG	JGUG G CACCCAC	G 3930	CGTGGGTG GGCTAGCTACAACGA CACACGCT	5216

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2301 CGUGUG	GC A CCCACGAU	3931	ATCGTGGG GGCTAGCTACAACGA GCCACACG 5217
	CC A CGAUCACA		TGTGATCG GGCTAGCTACAACGA GGGTGCCA 5218
	CG A UCACAGGA		TCCTGTGA GGCTAGCTACAACGA CGTGGGTG 5219
	UC A CAGGAAAC	-	GTTTCCTG GGCTAGCTACAACGA GATCGTGG 5220
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	GA A UCAGACGA		TCGTCTGA GGCTAGCTACAACGA TCTCCAGG 5222
	CAG A CGACAAGU		ACTTGTCG GGCTAGCTACAACGA CTGATTCT 5223
<u> </u>	ACG A CAAGUAUU		AATACTTG GGCTAGCTACAACGA CGTCTGAT 5224
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	CUC A UGCACGGC		GCCGTGCA GGCTAGCTACAACGA GAGACTTC 5230
	CAU G CACGGCAU		ATGCCGTG GGCTAGCTACAACGA ATGAGACT 5231
	UGC A CGGCAUCU		AGATGCCG GGCTAGCTACAACGA GCATGAGA 5232
	ACG G CAUCUGGO		CCCAGATG GGCTAGCTACAACGA CGTGCATG 5233
	GGC A UCUGGGA		TTCCCAGA GGCTAGCTACAACGA GCCGTGCA 5234
	GGA A UCCCCCU		GAGGGGGA GGCTAGCTACAACGA TCCCAGAT 5235
	UCC A CAGAUCAT		ATGATCTG GGCTAGCTACAACGA GGAGGGGG 5236
	CAG A UCAUGUGO		CCACATGA GGCTAGCTACAACGA CTGTGGAG 5237
	AUC A UGUGGUUT		AAACCACA GGCTAGCTACAACGA GATCTGTG 5238
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	IGUG G UUUAAAG		TCTTTAAA GGCTAGCTACAACGA CACATGAT 5240
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	AUA A UGAGACC		GGGTCTCA GGCTAGCTACAACGA TATCTTTA 5242
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	CUU G UAGAAGA		GTCTTCTA GGCTAGCTACAACGA AAGGGTCT 5244
	BAAG A CUCAGGC		TGCCTGAG GGCTAGCTACAACGA CTTCTACA 5245
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	AUCC G CAGAGUG		TCACTCTG GGCTAGCTACAACGA GGATAGTG 5255
	CAGA G UGAGGAA		CTTCCTCA GGCTAGCTACAACGA TCTGCGGA 5256
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	GAAG G CCUCUA		TGTAGAGG GGCTAGCTACAACGA CTTCGTCC 5258
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	CUAC A CACCOGC		CTGGCAGG GGCTAGCTACAACGA GTAGAGGC 5260
	ACCU G CCAGGC		ATGCCTGG GGCTAGCTACAACGA AGGTGTAG 5261
	CCAG G CAUGCAG		ACTGCATG GGCTAGCTACAACGA CTGGCAGG 5262
	AGGC A UGCAGU		ACACTGCA GGCTAGCTACAACGA GCCTGGCA 5263
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2545	GUGCAAAA G UGGAGGCA	3984	TGCCTCCA GGCTAGCTACAACGA TTTTGCAC	5270
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	GCACGGCG G UGAUUGCC	4001	CATGGCAA GGCTAGCTACAACGA CACCGCCG	5287
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—— —	AUCAUCCU A CGGACCGU		ACGGTCCG GGCTAGCTACAACGA AGGATGAT	5297
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270	2 GAAGACAG G CUACUUGU	4018	ACAAGTAG GGCTAGCTACAACGA CTGTCTTC	
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271	3 ACUUGUCC A UCGUCAUC	4021	CATGACGA GGCTAGCTACAACGA GGACAAGT	-}
271	6 UGUCCAUC G UCAUGGAU	J 4022	ATCCATGA GGCTAGCTACAACGA GATGGACA	· · · · · · · · · · · · · · · · · · ·
271	9 CCAUCGUC A UGGAUCC	4023	TGGATCCA GGCTAGCTACAACGA GACGATGG	
272	3 CGUCAUGG A UCCAGAUG	3 4024	CATCTGGA GGCTAGCTACAACGA CCATGACG	
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274	4 CCCAUUGG A UGAACAU	U 4028	AATGTTCA GGCTAGCTACAACGA CCAATGGG	5314
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2773 CUUAUGAU G	CCAGCAAA 4037	TTTGC	TGG	GGCTAGCTACAACG	A ATCATAAG	5323
2777 UGAUGCCA G		CCCAT	TTG	GGCTAGCTACAACG	A TGGCATCA	5324
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			ACGG	GGCTAGCTACAACG		5332
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	CCUUUGGC 4049					
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2910 GUCAAAAU (7 TCT	TTCA	A GGCTAGCTACAA	CGA ATTTTGA	C 5353
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2926 AAGGAGCA				G GGCTAGCTACAA		
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	GGGGCACG A UUCCGUCA		TGACGGAA GGCTAGCTACAACGA CGTGCCCC 5402
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 	4 GUCCCUCA G UGAUGUA		CTACATCA GGCTAGCTACAACGA TGAGGGAC 5426
ļ	7 CCUCAGUG A UGUAGAA		CTTCTACA GGCTAGCTACAACGA CACTGAGG 5427
	59 UCAGUGAU G UAGAAGA		CITCHEST COUNTY ON A CONTROL FACE
32	SOUCHOUGHU G UNGRAGA	4147	

2274 ANGAGGAA G CUCCUGAA 4144 TRYACRGA GGCTAGCTACAACGA TYCCTCTT 5429 2284 UCCUGAAG A ULUGUAUA 4144 TATACAGA GGCTAGCTACAACGA ACAGATCT 5431 2286 GARAQUCU G UAUAAGGA 4145 TCCTTATA GGCTAGCTACAACGA AGATCTT 5431 2296 GUAUAAGGA CUUCCUGA 4146 ASTCCTTA GGCTAGCTACAACGA AGATCTT 5432 2396 GUAUAAGGA CUUCCUGA 4146 ASTCCTTA GGCTAGCTACAACGA ACAGATCT 5432 2396 GUAUAAGGA CUUCCUGA 4146 ASTCCTTA GGCTAGCTACAACGA CACTATTAC 5433 3304 AUGUCUGGA CUUCCUGA 4149 ATGAGAAG GGCTAGCTACAACGA CACTATTAC 5433 3314 CUUGAGGA GUCUCCUGU 4150 AGATGAGA GGCTAGCTACAACGA CCCCAAG 5436 3314 CUUGAGGA GUCUCCUGU 4151 GATACAGA GGCTAGCTACAACGA GCCCAAG 5436 3326 AUGUCUGU 4151 GATACAGA GGCTAGCTACAACGA GCCCAAG 5436 3326 AUGUGUGU A 152 AGCTTATA GGCTAGCTACAACGA GACAGATC 5437 3326 CAUGUGU A 154 CTTGGAAG GGCTAGCTACAACGA AGATGAGA 5438 3336 GUCUCAAG G CUUCCAAG 4154 CTTGGAAG GGCTAGCTACAACGA AGATGAGA 5438 3337 GCUUCCAA G UUGUGAGA 4155 CTTAGCA GGCTAGCTACAACGA ACAGAGTC 5440 3337 GGUUCCAA G UUGUGGAC 4158 GACCTTACA GGCTAGCTACAACGA ACAGAGTC 5441 3340 UCCAAGGG C UUCUUGGC 4158 GACCTCA GGCTAGCTACAACGA ACAGAGTC 5443 3340 UCCAAGGG G UUCUUGGC 4158 GACCTCACAG GGCTAGCTACAACGA CCTTAGGA 5442 3345 GCCAAGGA G UUCUUGGC 4158 GACCTCACAGGA GCCTAGCTACAACGA CCTTAGGA 5443 3346 UCCAGAGA G UUCUUGGC 4158 GACCTCACAGGA GCCTAGCTACAACGA CCTTAGGC 5443 3346 UCCAGAGA 4161 TTTGGGA GGCTAGCTACAACGA CCTTAGC 5443 3346 UCCAGAGA 4162 TTTGGGA GGCTAGCTACAACGA CCTTAGC 5445 3346 UCCAGAGA 4162 TTTGGGA GGCTAGCTACAACGA GCTAGCTACAACGA CCCTTAGC 5446 3372 UCGCGAAA G UUGUACA 4163 TTTGGGA GGCTAGCTACAACGA GCTAGCTACAACGA GCTAGCTACAACGA CCCTTAGGA 5446 3374 UCGAGAAG CUUCUCCA 4164 TTTGGGA GGCTAGCTACAACGA GCTAGCTTACAACGA CCCTGTGG 5449 3346 UCGAGAGA 4168 TTTGGGA GGCTAGCTACAACGA GC				141	
2888 GARGARICU G UNLANGGA 4145 3290 GARUCUGU A UNAGGACU 4146 3290 GARUCUGU A CUUGGAG 4147 17 CAGGARA GGCTAGCTACAACGA CACARATTC 5432 3290 GUNUAAGG A CUUCUGU 4149 3314 ACUUGGA C CUUCUUU 4150 3314 ACUUGGA C AUCUCUU 4151 3314 ACUUGGA C AUCUCUU 4151 3314 ACUUGGA C AUCUCUU 4151 3314 ACUUGGAC A UUCCAAC 3315 MACAUCUC A UCUGUAL 4149 3315 MACAUCUC A UCUGUAL 4151 3323 UCUCAUCU A UNACAGCU 4152 3326 CAUCUCU A UUCCAAC 3327 CUUCCAAC 3328 CACARACACA CCTATACACACA CACARATTC 5433 3329 CUUCUACA C UUCCAAC 3154 CTATACACACACACACACACACACACACACACACACACA	3274 A	AGAGGAA G CUCCUGAA	4143	TTCAGGAG GGCTAGCTACAACGA TTCCTCTT	5429
2820 ABRIGUEGE A UNAGRACU 4146 ARTCOTTA GSCTARGATACAACGA ACAGATCT 5432 2826 GUNUARAG A CUUCCUGA 4147 TOAGGAAG GSCTAGCTACAACGA CCTTATAC 5433 3826 ACUUCCUGA A CUUCCUGA 4147 TOAGGAAG GSCTAGCTACAACGA CCTTATAC 5433 3812 ACCUUGGA C CUUCCUGA 4149 ANGAGATG GSCTAGCTACAACGA CCCTATAC 5435 3814 CUUGGAGC A UCUCCAUCU 4149 ANGAGATG GSCTAGCTACAACGA TCCAAGGT 5435 3819 ACCUUCCA UCUCGUUCC 4150 AGATGAGG GGCTAGCTACAACGA GCTCCAAGG 5436 3819 ACCUUCCU G UUCCACGC 4152 AGATGAGG GGCTAGCTAACGA AGATGAGG 5436 3826 CAUCUGGUU A CAGCUUCC 4151 GARACAGG GGCTAGCTAACGA AGATGAGG 5438 3829 CUGUUCCAA G CUUCCAAG 4154 ATTCGAAG GGCTAGCTACAACGA AGATGAGG 5439 3829 CUGUUCCAA G CUUCCAAG 4154 CTTCGAAG GGCTAGCTACAACGA AGATGAGG 5440 3837 GCUUCCAA G CUUCCAAG 4154 CTTCGAAG GGCTAGCTACAACGA AGACGATC 5440 38340 CUCAAGGG C CUAGAGGC 4155 CTTAGCAA GGCTAGCTACAACGA TCTAGACG 5440 38341 GCUAAGGG C AUGGAGAU 4155 ACTCCATA GGCTAGCTACAACGA CACTAGCC 5443 38342 GCAAAGGG C AUGGAGAU 4155 ACTCCATA GGCTAGCTACAACGA CACTAGC 5441 38343 GCAAAGGG C AUGGAGAU 4156 GAACTCCA GGCTAGCTACAACGA CACTAGCC 5443 38361 AGUUCUUG C CAUCACGA 4161 TTTCGCGA GGCTAGCTACAACGA CACAGAAC 5446 38363 UUGUUGGC A UCUCCACA 4164 TTTCGCGA GGCTAGCTACAACGA ACCAGAC 5446 38376 CUGUGGAA G UCUCCACA 4164 TTTCGCGA GGCTAGCTACAACGA ACCAGAC 5446 38376 CUGCGAAAGU G UAUCCACA 4164 TTTCGCGA GGCTAGCTACAACGA ACCAGAA 5447 38376 CUGCGAAAGU G UAUCCACA 4164 TTTCGCGA GGCTAGCTACAACGA ACCATACCA 5460 38386 CUGCGAGG A CUGGGGG 4166 TCGTCCCG GGCTAGCTACAACGA ACCATTCCC 5453 38396 GUAGACGA C 4166 GTTCCTT GGCTAGCTACAACGA ACCATTCCC 5453 38396 CUGCGGGG A CCUGGGGG 4167 CCGCCAGG GGCTAGCTACAACGA ACCATTTC 5451 38391 ACCUCUU A UCCACAA 4164 TTTCGTGG GGCTAGCTACAACGA ACCATTTC 5451 38391 ACCUCUCU A UCCACAA 4164 TTTCGTGG GGCTAGCTACAACGA ACCATTTC 5451 38391 ACCUCUCU A UCCACAA 4164 TTTCTCGG GGCTAGCTACAACGA ACCATTT 5451 38496 CUGGGGG A CGGAGAGA 4166 TCGTCCCG GGCTAGCTACAACGA ACCATTT 5451 38496 CUGGGGG A CGGAGAAUAU 4167 AACCAGG	3284 U	CCUGAAG A UCUGUAUA	4144	TATACAGA GGCTAGCTACAACGA CTTCAGGA	5430
2926 [GURIDARGE A CUUCCUGA] 4147 TCAGGARG GGCTAGCTACANCGA CCTTATAC 5433 3314 [ACUUCCUG A CCUUGGAG 4148 CTCCAAGG GGCTAGCTACANCGA CCTAATAC 5434 3312 [ACCUUGA G CAUCCCAU 4149 ATGRATIS GGCTAGCTACANCGA CCAAGGOT 5435 3314 [ACUUGGAGC A UCUCUNICU 4150 AGATGAGA GGCTAGCTACANCGA CCCAAGGOT 5436 3314 [ACUUGGAGC A UCUCUNICU 4150 AGATGAGA GGCTAGCTACANCGA GCTCCAAGG 5436 3314] AGACUUCC A UCUGUUCC 4151 GTAACAGA GGCTAGCTACANCGA AGATGAGA 5436 3315] AGACUUCC A UCUGUUCC 4151 GTAACAGA GGCTAGCTACANCGA AGATGAGA 5437 3326 [CAUCUGUU A CAGCUUCC 4153] GGAAGCTG GGCTAGCTACANCGA AGATGAGA 5439 3327 [CUUCCANCU 6 UDACAGCU 4152 AGCTGATA GGCTAGCACAGA AGATGAGA 5439 3328 [CUUCCANCU 6 CUACAGAG 4154 CTTGGARA GGCTAGCTACANCGA AGATGAGA 5440 3340 [UCCAAGUG 6 UCUACAGG 4155 CTTAGCA GGCTAGCTACANCGA TAGGAAGC 5441 3340 [UCCAAGUG 6 CUAAAGGC 4155 CTTAGCA GGCTAGCTACANCGA TAGGAAGC 5441 3347 [GUCAAGG 6 CAUGCGAC 4155 GCCCTTAG GGCTAGCTACAACGA CACTTAGA 5442 3349 [CUAAGGC A UGCAGCAU 4155 ACTCCAT GGGTAGCTACAACGA CACTTAGC 5443 3349 [CUAAGGC A UGCACCA 4154] GAACTCCA GGCTAGCTACAACGA CACTTAGC 5443 3354 [GCAUGGA 6 UUCUUGGC 4159] GCCAAGAA GGCTAGCTACAACGA TCCATCC 5445 3363 UUCUUGGC C CAAAAGU 4152 GCCATGAA GGCTAGCTACAACGA CACTTACC 5445 3364 UUCUGGC A UCCACCAG 4162 TCACCAC GGCTAGCTACAACGA GCCTACCC 5445 3374 [GCAAAAGU 6 UUCUCCAC 4164 TTTCGCGA GGCTAGCTACAACGA GCCTACCCA 5448 3374 [GCAAAAGU 6 UUCCACCA 4164 TTTCGCGA GGCTAGCTACAACGA ACCACTTAC 3376 [GAAAAGU 6 UUCCACCA 4164 TTTCGCGA GGCTAGCTACAACGA ACCACTTC 5449 3377 [GCAAAAAU 6 UUCCACCA 4164 TTTCGCGA GGCTAGCTACAACGA ACCACTTC 5449 3378 [GCAAGAAGU 6 UUCCACCA 4164 TTTCGCGA GGCTAGCTACAACGA ACCACTTC 5449 3379 [ACAGGACGA ACCACGA 4165 GTACCCAC ACGAACGA GACTACTC 5459 3391 [ACCUGCA A CCACGAGG 4165 CCTTTGGA GGCTAGCTACAACGA ACCACTTC 5451 3391 [ACCUGCA A CCACGAGG 4165 CCTTTGGA GGCTAGCTACAACGA ACCACTTC 5451 3391 [ACCUGCA A UCCACCA 4166 GTACCCAC GGCTAGCTACAACGA ACCACTTC 5451 3391 [ACCUGCA A UCCACCA 4166 GTACCCAC GGCTAGCTACAACGA ACCACTTC 5451 3391 [ACCUGCA A UCCACCA 4166 GTACCCAC GGCTAGCTACAACGA ACCACTTC 5451 3403 [CAGAAAU A UCCACCA 4166 GTACCCAC GGC	3288 G	AAGAUCU G UAUAAGGA	4145	TCCTTATA GGCTAGCTACAACGA AGATCTTC	5431
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3314 ACUUCUG A CCUUGGAG 4148 CTCCANGG GGCTAGCTACAACGA CAGGAAGT 5434 3312 ACUUGGA G CAUCUCU 4150 ARGARGAG GGCTAGCTACAACGA TCCAAGG 5435 3314 CUUGAGAC A UCUCULUC 4151 GTAACAGA GGCTAGCTACAACGA GGCTACAACGA GCTCCAAG 5436 3319 AGAUCUC A UCUGUURC 4151 GTAACAGA GGCTAGCTACAACGA GAGTAGCA 5437 3323 UCUCALUC 4 UUACACCU 4152 GGAAGCTG GGCTAGCTACAACGA AGAGGATG 5439 3326 CAUCUGU A CAGCUUCC 4153 GGAAGCTG GGCTAGCTACAACGA AACAGGATG 5439 3326 CAUCUGU A CAGCUUCC 4154 GCTTGGAAG GGCTAGCTACAACGA AACAGGATG 5439 3329 CUUUUCAA G CUUCCAAG 4154 CTTGGAAG GGCTAGCTACAACGA AACAGGATG 5440 3337 GCUUCCAA G UGGGUAGA 4156 GCCCTTAG GGCTAGCTACAACGA CACTTGGA 5441 3340 UCCAAGUG G CUUGAGGC 4156 GCCCTTAG GGCTAGCTACAACGA CACTTGGA 5442 3347 GCUAAGGG G UUUUGGG 4157 ACTCCATG GGCTAGCTACAACGA CACTTGGA 5442 3349 UUAAGGGC A UUGAGGGU 4158 GAACTCCA GGCTAGCTACAACGA CCCTTAGC 5443 3346 GCCAGGGA G UUCUUGGC 4158 GAACTCCA GGCTAGCTACAACGA CCCTTAGC 5445 3361 AGUUCUUG G CAUCGGA 4160 TCGCGATG GGCTAGCTACAACGA CCCATGCC 5445 3361 AGUUCUUG G CAUCGGA 4161 TTTGGGG GGCTAGCTACAACGA CCCATGCC 5445 3361 AGUUCUUG G CAUCGGA 4161 TTTGGGG GGCTAGCTACAACGA CCCATGCC 5445 3366 UUGUGGC A UUGCGGAA 4161 TTTGGGG GGCTAGCTACAACGA CCCATGCC 5446 3366 UUGCGCA 4164 TTTGGGG GGCTAGCTACAACGA CCCAAGGA 5447 3374 GAAAAGU G UAUCCACA 4164 TTTGGGG GGCTAGCTACAACGA ACTTTCG GGCTAGCTACAACGA CCCAAGGA 5449 3374 GAAAAGU G UAUCCACA 4164 TTTGGGG GGCTAGCTACAACGA ACTTTCG GGCTAGCTACAACGA CACTTTC 5451 3386 CUGGCGCG A CGAAAGU 4165 TCGTGCG GGCTAGCTACAACGA ACTTTCG GGCTAGCTACAACGA CACTTTC 5451 3391 GGGCCCU G CAGGACA A UAUCCCCU 4164 TTTGGGG GGCTAGCTACAACGA CCCTTGG 5453 3391 GGGCAGG A CGAAAGU 4164 TTTGGGG GGCTAGCTACAACGA CCCTGTG 5453 3391 GGGCAGG A CGAAAGU 4166 GGTCCCTG GGCTAGCTACAACGA CCCTGTG 5453 3391 GGGCAGG A CGAAAGU 4164 TTTGGGG GGCTAGCTACAACGA CCCCTGTG 5453 3401 GGCCAGGA A			4147	TCAGGAAG GGCTAGCTACAACGA CCTTATAC	5433
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3411 AUCCUCUU A UCGGAGAA 4173 TTCTCCGA GGCTAGCTACAACGA AAGAGGAT 5459 3422 GGAGAAGA A CGUGGUUA 4174 TAACCACG GGCTAGCTACAACGA TCTTCTCC 5460 3424 AGAAGAAC G UGGUUAAA 4175 TTTAACCA GGCTAGCTACAACGA CTCTTCT 5461 3427 AGAACGUG G UUAAAAUC 4176 GATTTTAA GGCTAGCTACAACGA CACGTTCT 5462 3433 UGGUUAAA A UCUGUGAC 4177 GTCACAGA GGCTAGCTACAACGA CACGTTCT 5463 3437 UAAAAUCU G UGACUUUG 4178 CAAAGTCA GGCTAGCTACAACGA AGATTTTA 5464 3440 AAUCUGUG A CUUUGGCU 4179 AGCCAAAG GGCTAGCTACAACGA CACAGATT 5465 3446 UGACUUUG G CUUGGCCC 4180 GGGCCAAG GGCTAGCTACAACGA CACAGATT 5465 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CACAGATC 5467 3458 GGCCCGGG A UAUUUAUAA 4182 TATAAATA GGCTAGCTACAACGA CCCGGGCC 5468 3460 CCCGGGAU A UUUAUAAA 4183 TTTATAAA GGCTAGCTACAACGA CCCGGGCC 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA ATCCCGGG 5469 3470 UUAUAAAG A UCCAGAUU 4185 AATCTGGA GGCTAGCTACAACGA CTTTATAA 5471 3476 AGAUCCAG A UUAUGUCA 4186 TGACATAA GGCTAGCTACAACGA CTTTATAA 5471 3476 AGAUCCAG A UUAUGUCA 4186 TGACATAA GGCTAGCTACAACGA CTTGGATCT 5472 3479 UCCAGAUU A UGUCAGAA 4187 TTCTGACA GGCTAGCTACAACGA ATCTCGA 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTCTGAC GGCTAGCTACAACGA ATCTCGA 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTCTGAC GGCTAGCTACAACGA ATCTCGT 5476 3494 AAAAGGAG A UGCUCGCC 4189 GGCGAGCA GGCTAGCTACAACGA ATCTCCTT 5476 3496 AAGGAGAU G CUCGCCU 4190 GAGGCGAG GGCTAGCTACAACGA ATCTCCTT 5476 3500 AGAUGCUC G CCUCCCUU 4191 AAGGGAGG GGCTAGCTACAACGA ATCTCCTT 5476 3513 CCUUUGAA A UGGCCCCA 4193 TGGGGCCA GGCTAGCTACAACGA CCATTTCA 5479 3520 AAUGGAUG G CCCCAGAA 4193 TGGGGCCA GGCTAGCTACAACGA CATCCATT 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CATCCATT 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CATCCATT 5479	3401	GGCACGAA A UAUCCUCU	4171	AGAGGATA GGCTAGCTACAACGA TTCGTGCC	5457
3422 GGAGAAGA A CGUGGUUA 4174 TAACCACG GGCTAGCTACAACGA TCTTCTCC 5460 3424 AGAAGAAC G UGGUUAAA 4175 TTTAACCA GGCTAGCTACAACGA GTTCTTCT 5461 3427 AGAACGUG G UUAAAAUC 4176 GATTTTAA GGCTAGCTACAACGA CACGTTCT 5462 3433 UGGUUAAA A UCUGUGAC 4177 GTCACAGA GGCTAGCTACAACGA CACGTTCT 5463 3437 UAAAAUCU G UGACUUUG 4178 CAAAGTCA GGCTAGCTACAACGA AGATTTTA 5464 3440 AAUCUGUG A CUUUGGCU 4179 AGCCAAAG GGCTAGCTACAACGA CACAGATT 5465 3446 UGACUUUG G CUUGGCCC 4180 GGGCCAAG GGCTAGCTACAACGA CACAGATT 5466 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CAAAGTCA 5467 3458 GGCCCGGG A UAUUUAUAA 4182 TATAAATA GGCTAGCTACAACGA CCCGGGCC 5468 3460 CCCGGGAU A UUUAUAAA 4183 TTTATAAA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTACAACGA ATCCCGGG 5469 3470 UUAUAAAG A UCCAGAUU 4185 AATCTGGA GGCTAGCTACAACGA CTTATAA 5471 3476 AGAUCAG A UUAUGUCA 4186 TGACATAA GGCTAGCTACAACGA CTGGATCT 5472 3479 UCCAGAUU A UGUCAGAA 4187 TTCTGACA GGCTAGCTACAACGA ATCTCGG 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTTCTGACA GGCTAGCTACAACGA ATCTCGG 5473 3494 AAAAGGAG A UGCUCGCC 4189 GGCGAGCA GGCTAGCTACAACGA ATCTCGT 5476 3496 AAGGAGAU G CUCGCCUC 4190 GAGGCGAG GGCTAGCTACAACGA ATCTCCTT 5476 3513 CCUUUGAA A UGGAUGGC 4192 GCCATCCA GGCTAGCTACAACGA CCATTTCA 5477 3513 CCUUUGAA A UGGCCCCA 4191 TGGGGCCA GGCTAGCTACAACGA CCATTCCTT 5476 3520 AAUGGAUG G CCCCAGAA 4191 TGGGGCCA GGCTAGCTACAACGA CCATTCCAT 5477 3513 CCUUUGAA A UGGCCCCA 4191 TGGGGCCA GGCTAGCTACAACGA CCATTCCAT 5477 3520 AAUGGAUG G CCCCAGAA 4191 TGGGGCCA GGCTAGCTACAACGA CCATTCCAT 5477	3403	CACGAAAU A UCCUCUUA	4172	TAAGAGGA GGCTAGCTACAACGA ATTTCGTG	5458
3424 AGAAGAAC G UGGUUAAA 4175 TTTAACCA GGCTAGCTACAACGA GTTCTTCT 5461 3427 AGAACGUG G UUAAAAUC 4176 GATTTTAA GGCTAGCTACAACGA CACGTTCT 5462 3433 UGGUUAAA A UCUGUGAC 4177 GTCACAGA GGCTAGCTACAACGA TTTAACCA 5463 3437 UAAAAUCU G UGACUUUG 4178 CAAAGTCA GGCTAGCTACAACGA AGATTTTA 5464 3440 AAUCUGUG A CUUUGGCCU 4179 AGCCAAG GGCTAGCTACAACGA AGATTTTA 5465 3446 UGACUUUG G CUUGGCCC 4180 GGGCCAAG GGCTAGCTACAACGA CACAGATT 5465 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CAAAGTCA 5466 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CAAGCCAA 5467 3458 GGCCCGGG A UAUUUAUAA 4182 TATAAATA GGCTAGCTACAACGA CCCGGGCC 5468 3460 CCCGGGAU A UUUAUAAA 4183 TTTATAAA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA ATCCCGGG 5469 3470 UUAUAAAG A UCCAGAUU 4185 AATCTGGA GGCTAGCTACAACGA ATATCC 5470 3470 UUAUAAAG A UCCAGAUU 4185 AATCTGGA GGCTAGCTACAACGA CTGGATCT 5472 3479 UCCAGAUU A UGUCAGAA 4186 TGACATAA GGCTAGCTACAACGA ATCTGGA 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTCTGACA GGCTAGCTACAACGA ATAATCTG 5474 3494 AAAAGGAG A UGCUCGCC 4189 GGCGAGCA GGCTAGCTACAACGA ATAATCTG 5476 3496 AAGGAGAU G CUCGCCUC 4190 GAGGCGAG GGCTAGCTACAACGA ATCCCTT 5476 3500 AGAUGCUC G CCUCCCUU 4191 AAGGGAGG GGCTAGCTACAACGA ATCCCTT 5476 3513 CCUUUGAA A UGGAUGGC 4192 GCCATCCA GGCTAGCTACAACGA CTCCTTT 5476 3510 UGAAAUGG A UGGCCCCA 4193 TGGGGCCA GGCTAGCTACAACGA CTCCATT 5479 3520 AAUGGAUG G CCCCAGAA 4193 TGGGGCCA GGCTAGCTACAACGA CATCCATT 5480	3411	AUCCUCUU A UCGGAGAA	4173	TTCTCCGA GGCTAGCTACAACGA AAGAGGAT	5459
3427 AGAACGUG G UUAAAAUC 4176 GATTTTAA GGCTAGCTACAACGA CACGTTCT 5462 3433 UGGUUAAA A UCUGUGAC 4177 GTCACAGA GGCTAGCTACAACGA TTTAACCA 5463 3437 UAAAAUCU G UGACUUUG 4178 CAAAGTCA GGCTAGCTACAACGA AGATTTTA 5464 3440 AAUCUGUG A CUUUGGCU 4179 AGCCAAAG GGCTAGCTACAACGA CACAGATT 5465 3446 UGACUUUG G CUUGGCCC 4180 GGGCAAG GGCTAGCTACAACGA CAAAGTCA 5466 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CAAAGTCA 5467 3458 GGCCCGGG A UAUUUAUA 4182 TATAAATA GGCTAGCTACAACGA CCCGGGCC 5468 3460 CCCGGGAU A UUUAUAAA 4183 TTTATAAA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA ATATCC 5470 3470 UUAUAAAG A UCCAGAUU 4185 AATCTGGA GGCTAGCTACAACGA CTTTATAA 5471 3476 AGAUCCAG A UUAUGUCA 4186 TGACATAA GGCTAGCTACAACGA CTGGATCT 5472 3479 UCCAGAUU A UGUCAGAA 4187 TTCTGACA GGCTAGCTACAACGA ATCCTGGA 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTTCTGACA GGCTAGCTACAACGA ATCCTGGA 5473 3494 AAAAGGAG A UGCUCGCC 4189 GGCGAGCA GGCTAGCTACAACGA ATCCTCTT 5476 3496 AAGGAGAU G CUCGCCUC 4190 GAGGCGAG GGCTAGCTACAACGA ATCCTCTT 5476 3500 AGAUGCUC G CCUCCCUU 4191 AAGGGAGG GGCTAGCTACAACGA ATCTCCTT 5476 3513 CCUUUGAA A UGGAUGGC 4192 GCCATCCA GGCTAGCTACAACGA CTCTTTC 5479 3520 AAUGGAUG G CCCCAGAA 4193 TGGGGCCA GGCTAGCTACAACGA CATCTCTT 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CATCTCTT 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CATCCTTT 5479	3422	GGAGAAGA A CGUGGUUA	4174	TAACCACG GGCTAGCTACAACGA TCTTCTCC	5460
3437 UGGUUAAA A UCUGUGAC 4177 GTCACAGA GGCTAGCTACAACGA TTTAACCA 5463 3437 UAAAAUCU G UGACUUUG 4178 CAAAGTCA GGCTAGCTACAACGA AGATTTTA 5464 3440 AAUCUGUG A CUUUGGCU 4179 AGCCAAAG GGCTAGCTACAACGA CACAGATT 5465 3446 UGACUUUG G CUUGGCCC 4180 GGGCCAAG GGCTAGCTACAACGA CAAAGTCA 5466 3451 UUGGCUUG G CCCGGGAU 4181 ATCCCGGG GGCTAGCTACAACGA CAAGCCAA 5467 3458 GGCCCGGG A UAUUUAUA 4182 TATAAATA GGCTAGCTACAACGA CCCGGGCC 5468 3460 CCCGGGAU A UUUAUAAA 4183 TTTATAAA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA ATCCCGGG 5469 3464 GGAUAUUU A UAAAGAUC 4184 GATCTTTA GGCTAGCTACAACGA CTTTATAA 5471 3476 AGAUCCAG A UUAUGUCA 4186 TGACATAA GGCTAGCTACAACGA CTGGATCT 5472 3479 UCCAGAUU A UGUCAGAA 4187 TTCTGACA GGCTAGCTACAACGA AATCTCGG 5473 3481 CAGAUUAU G UCAGAAAA 4188 TTTTCTGAC GGCTAGCTACAACGA ATAATCTG 5474 3494 AAAAGGAG A UGCUCGCC 4189 GGCGAGCA GGCTAGCTACAACGA ATAATCTG 5476 3500 AGAUGCUC G CCUCCCUU 4191 AAGGGAGG GGCTAGCTACAACGA ATCTCCTT 5476 3513 CCUUUGAA A UGGACCCA 4193 TGGGGCCA GGCTAGCTACAACGA CCATTTCA 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CCATTTCA 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CCATTTCA 5479 3520 AAUGGAUG G CCCCAGAA 4194 TTCTGGGG GGCTAGCTACAACGA CCATTTCA 5479	3424	AGAAGAAC G UGGUUAAA	4175	TTTAACCA GGCTAGCTACAACGA GTTCTTCT	5461
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3715 UGUACCAG A CCAUGCUG 4235 CAGCATGG GGCTAGCTACAACGA CTGGTACA 5521 3718 ACCAGACC A UGCUGGAC 4236 GTCCAGCA GGCTAGCTACAACGA GGTCTGGT 5522 3720 CAGACCAU G CUGGACUG 4237 CAGTCCAG GGCTAGCTACAACGA ATGGTCTG 5523 3725 CAUGCUGG A CUGCUGGC 4238 GCCAGCAG GGCTAGCTACAACGA CCAGCATG 5524 3728 GCUGGACU G CUGGCACG 4239 CGTGCCAG GGCTAGCTACAACGA AGTCCAGC 5525 3732 GACUGCUG G CACGGGGA 4240 TCCCCGTG GGCTAGCTACAACGA CAGCAGTC 5526 3734 CUGCUGGC A CGGGGAGC 4241 GCTCCCCG GGCTAGCTACAACGA GCCAGCAG 5527 3741 CACGGGGA G CCCAGUCA 4242 TGACTGGG GGCTAGCTACAACGA TCCCCGTG 5528 3746 GGAGCCCA G UCAGAGAC 4243 GTCTCTGA GGCTAGCTACAACGA TGGGCTCC 5529 3753 AGUCAGAG A CCCACGUU 4244 AACGTGGG GGCTAGCTACAACGA CTCTGACT 5530 3757 AGAGACCC A CGUUUUCA 4245 TGAAAACG GGCTAGCTACAACGA GGGTCTCT 5531 3759 AGACCCAC G UUUUCAGA 4246 TCTGAAAA GGCTAGCTACAACGA GTGGGTCT 5532 3768 UUUUCAGA G UUGGUGGA 4247 TCCACCAA GGCTAGCTACAACGA TCTGAAAA 5533)	
3715 UGUACCAG A CCAUGCUG 4235 3718 ACCAGACC A UGCUGGAC 4236 GTCCAGCA GGCTAGCTACAACGA GGTCTGGT 5522 3720 CAGACCAU G CUGGACUG 4237 3725 CAUGCUGG A CUGCUGGC 4238 GCCAGCAG GGCTAGCTACAACGA ATGGTCTG 5524 3728 GCUGGACU G CUGGCACG 4239 CGTGCCAG GGCTAGCTACAACGA AGTCCAGC 5525 3732 GACUGCUG G CACGGGGA 4240 TCCCCGTG GGCTAGCTACAACGA CAGCAGTC 5526 3734 CUGCUGGC A CGGGGAC 4241 GCTCCCCG GGCTAGCTACAACGA GCCAGCAG 5527 3741 CACGGGGA G CCCAGUCA 4242 TGACTGGG GGCTAGCTACAACGA TCCCCGTG 5528 3746 GGAGCCCA G UCAGAGAC 4243 GTCTCTGA GGCTAGCTACAACGA TGGGCTCC 5529 3753 AGUCAGAG A CCCACGUU 4244 AACGTGGG GGCTAGCTACAACGA CTCTGACT 5530 3757 AGAGACCC A CGUUUUCA 4245 TGAAAACG GGCTAGCTACAACGA GGGTCTCT 5531 3759 AGACCCAC G UUUUCAGA 4246 TCTGAAAA GGCTAGCTACAACGA GTGGGTCT 5532 3768 UUUUCAGA G UUGGUGGA 4247 TCCACCAA GGCTAGCTACAACGA TCTGAAAA 5533					
3718 ACCAGACC A UGCUGGAC 4238 GICCAGCA GGCTAGCTACAACGA ATGGTCTG 5523 3720 CAGACCAU G CUGGACUG 4237 CAGTCCAG GGCTAGCTACAACGA CCAGCATG 5524 3728 GCUGGACU G CUGGCACG 4239 CGTGCCAG GGCTAGCTACAACGA AGTCCAGC 5525 3732 GACUGCUG G CACGGGGA 4240 TCCCCGTG GGCTAGCTACAACGA CAGCAGTC 5526 3734 CUGCUGGC A CGGGGAGC 4241 GCTCCCCG GGCTAGCTACAACGA GCCAGCAG 5527 3741 CACGGGGA G CCCAGUCA 4242 TGACTGGG GGCTAGCTACAACGA TCCCCGTG 5528 3746 GGAGCCCA G UCAGAGAC 4243 GTCTCTGA GGCTAGCTACAACGA TGGGCTCC 5529 3753 AGUCAGAG A CCCACGUU 4244 AACGTGGG GGCTAGCTACAACGA CTCTGACT 5530 3757 AGAGACCC A CGUUUUCA 4245 TGAAAACG GGCTAGCTACAACGA GGGTCTCT 5531 3759 AGACCCAC G UUUUCAGA 4246 TCTGAAAA GGCTAGCTACAACGA GTGGGTCT 5532 3768 UUUUCAGA G UUGGUGGA 4247 TCCACCAA GGCTAGCTACAACGA TCTGAAAA 5533					
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3732 GACUGCUG G CACGGGA 4240 ICCCCGG GGCTAGCTACAACGA GCCAGCAG 5527 3734 CUGCUGGC A CGGGGAGC 4241 GCTCCCCG GGCTAGCTACAACGA GCCAGCAG 5527 3741 CACGGGGA G CCCAGUCA 4242 TGACTGGG GGCTAGCTACAACGA TCCCCGTG 5528 3746 GGAGCCCA G UCAGAGAC 4243 GTCTCTGA GGCTAGCTACAACGA TGGGCTCC 5529 3753 AGUCAGAG A CCCACGUU 4244 AACGTGGG GGCTAGCTACAACGA CTCTGACT 5530 3757 AGAGACCC A CGUUUUCA 4245 TGAAAACG GGCTAGCTACAACGA GGGTCTCT 5531 3759 AGACCCAC G UUUUCAGA 4246 TCTGAAAA GGCTAGCTACAACGA GTGGGTCT 5532 3768 UUUUCAGA G UUGGUGGA 4247 TCCACCAA GGCTAGCTACAACGA TCTGAAAA 5533					
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	37	72 CAGAGUUG G UGGAACAI	U 4248	ATGTTCCA GGCTAGCTACAACGA CAACTCTG	5534

		143	
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3799 UCUUGCAA G CUAAUGCU	4253	AGCATTAG GGCTAGCTACAACGA TTGCAAGA 5	539
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			547
3829 AAGACUAC A UUGUUCUU		ANGINICITY COOTILECTION	548
3832 ACUACAUU G UUCUUCCG		Cocariors, Cocariocation	5549
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3857 GACUUUGA G CAUGGAAG		CIICOINO GGOLINGELIA	5553
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3876 GAUUCUGG A CUCUCUCU		AGAGAGAS COCINGOLIGIZACES CONTRACTOR	5556
3885 CUCUCUCU G CCUACCUC		0.10011100 000111011101110111011	
3889 CUCUGCCU A CCUCACCU		MODICA COCCINEDATION	5557
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4015 GUGUAAAA A CAUUUGA		TCTTCAAA GGCTAGCTACAACGA GTTTTTAC	
4017 GUAAAAAC A UUUGAAC	GA 4301	TCTTCARA GGCIAGCIACAACGA GIIIIAC	1307

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	CTTCTAA GGCTAGCTACAACGA GGGATATC 5590
	ACTTCTGG GGCTAGCTACAACGA TCTTCTAA 5591
	FACTITTA GGCTAGCTACAACGA TTCTGGTT 5592
20-20 Michigan 6	TGGGATTA GGCTAGCTACAACGA TTTTACTT 5593
	ATCTGGGA GGCTAGCTACAACGA TACTTTTA 5594
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4070 AGAUGACA A COMMISSO 2522	ACTGTCCG GGCTAGCTACAACGA CTGGTTGT 5598
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40/3 CCAGACGG 11 CARGAGGT	TACCACTO GOCTAGOTAGOTAGO F.CO.
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4085 GGACAGUG G UAUGGUUC 4315	GAACCATA GOCTAGOTAGOTAGO ECON
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4217 AAGCGGCU A CCAGUCCG 4341	CGGACIGG GGCIACCIACCIA
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4255 ACACCACC G UGUACUCC 4352	GGAGTACA GGCTAGCTACAACGA GGTGGTGT 5638
4257 ACCACCGU G UACUCCAG 4353	CTGGAGTA GGCTAGCTACAACGA ACGGTGGT 5639
4259 CACCGUGU A CUCCAGUG 4354	CACTGGAG GGCTAGCTACAACGA ACACGGTG 5640
TEST CACCOUGU A COCCAGOO TSSA	

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4265 GU	JACUCCA G UGAGGAAG	4355	CTTCCTCA GGCTAGCTACAACGA TGGAGTAC 5641
4273 GU	GAGGAA G CAGAACUU	4356	AAGTTCTG GGCTAGCTACAACGA TTCCTCAC 5642
4278 GA	AGCAGA A CUUUUAAA	4357	TTTAAAAG GGCTAGCTACAACGA TCTGCTTC 5643
	JUUUAAA G CUGAUAGA	4358	TCTATCAG GGCTAGCTACAACGA TTTAAAAG 5644
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	UUGGAGU G CAAACCGG	4362	CCGGTTTG GGCTAGCTACAACGA ACTCCAAT 5648
	AGUGCAA A CCGGUAGC	4363	GCTACCGG GGCTAGCTACAACGA TTGCACTC 5649
<u></u>	CAAACCG G UAGCACAG		CTGTGCTA GGCTAGCTACAACGA CGGTTTGC 5650
<u> </u>	ACCGGUA G CACAGCCC		GGGCTGTG GGCTAGCTACAACGA TACCGGTT 5651
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	UAGCACA G CCCAGAUU		AATCTGGG GGCTAGCTACAACGA TGTGCTAC 5653
	AGCCCAG A UUCUCCAG		CTGGAGAA GGCTAGCTACAACGA CTGGGCTG 5654
	JUCUCCA G CCUGACUC		GAGTCAGG GGCTAGCTACAACGA TGGAGAAT 5655
			TCCCCGAG GGCTAGCTACAACGA CAGGCTGG 5656
	CAGCCUG A CUCGGGGA	ļ———	CAGTGTGG GGCTAGCTACAACGA CCCCGAGT 5657
	CUCGGGG A CCACACUG	 	GCTCAGTG GGCTAGCTACAACGA GGTCCCCG 5658
			GAGCTCAG GGCTAGCTACAACGA GTGGTCCC 5659
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4437	UUUGAAGU G UUGUUCU	U 4389	AAGAACAA GGCTAGCTACAACGA ACTTCAAA 5675
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TITE COMMINENT II CONTROL	GAGCTGCA GGCTAGCTACAACGA CATTTTCT 5811
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5223 CUGACUGC A CAAACCAG 4546	CTGGTTTG GGCTAGCTACAACGA GCAGTCAG 5832
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	GUUUUUU G UUUGGUAC	4627	GTACCAAA GGCTAGCTACAACGA AAAAAACA 5913
	UUGUUUG G UACCAUAG	4628	CTATGGTA GGCTAGCTACAACGA CAAACAAA 5914
	GUUUGGU A CCAUAGUG	4629	CACTATGG GGCTAGCTACAACGA ACCAAACA 5915
	UGGUACC A UAGUGUGA	4630	TCACACTA GGCTAGCTACAACGA GGTACCAA 5916
	UACCAUA G UGUGAAAU	4631	ATTTCACA GGCTAGCTACAACGA TATGGTAC 5917
<u></u>	CCAUAGU G UGAAAUGC	4632	GCATTTCA GGCTAGCTACAACGA ACTATGGT 5918
	GUGUGAA A UGCUGGGA		TCCCAGCA GGCTAGCTACAACGA TTCACACT 5919
	GUGAAAU G CUGGGAAC	4634	GTTCCCAG GGCTAGCTACAACGA ATTTCACA 5920
	IGCUGGGA A CAAUGACU		AGTCATTG GGCTAGCTACAACGA TCCCAGCA 5921
			TATAGTCA GGCTAGCTACAACGA TGTTCCCA 5922
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	HAACAAUG A CUAUAAGA		ATGTCTTA GGCTAGCTACAACGA AGTCATTG 5924
	CAAUGACU A UAAGACAU		ATAGCATG GGCTAGCTACAACGA CTTATAGT 5925
	ACUAUAAG A CAUGCUAU		CCATAGCA GGCTAGCTACAACGA GTCTTATA 5926
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	JAAGACAU G CUAUGGCA		ATGTGCCA GGCTAGCTACAACGA AGCATGTC 5928
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5680	UAUAGUCU G UUUAUGU	4650	TACATAAA GGCTAGCTACAACGA AGACTATA 5936
5684	GUCUGUUU A UGUAGAAA	4651	TTTCTACA GGCTAGCTACAACGA AAACAGAC 5937
5686	CUGUUUAU G UAGAAACI	4652	TGTTTCTA GGCTAGCTACAACGA ATAAACAG 5938
5692	AUGUAGAA A CAAAUGU	4653	TACATTTG GGCTAGCTACAACGA TTCTACAT 5939
5696	agaaacaa a uguaauai	J 4654	ATATTACA GGCTAGCTACAACGA TTGTTTCT 5940
5698	AAACAAAU G UAAUAUAI	J 4655	ATATATTA GGCTAGCTACAACGA ATTTGTTT 5941
5701	CAAAUGUA A UAUAUUA	A 4656	TTAATATA GGCTAGCTACAACGA TACATTTG 5942
5703	AAUGUAAU A UAUUAAA	G 4657	CTTTAATA GGCTAGCTACAACGA ATTACATT 5943
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5711	AUAUUAAA G CCUUAUA	U 4659	ATATAAGG GGCTAGCTACAACGA TTTAATAT 5945
5716	AAAGCCUU A UAUAUAA	U 4660	ATTATATA GGCTAGCTACAACGA AAGGCTTT 5946
5718	AGCCUUAU A UAUAAUG	A 4661	TCATTATA GGCTAGCTACAACGA ATAAGGCT 5947
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	UAUAUAUA A UGAACUU		AAAGTTCA GGCTAGCTACAACGA TATATATA 5949
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-	UGAACUUU G UACUAUU		GAATAGTA GGCTAGCTACAACGA AAAGTTCA 5951
	AACUUUGU A CUAUUCA		GTGAATAG GGCTÁGCTACAACGA ACAAAGTT 5952
	UUUGUACU A UUCACAU		AATGTGAA GGCTAGCTACAACGA AGTACAAA 5953
	UACUAUUC A CAUUUUG		ACAAAATG GGCTAGCTACAACGA GAATAGTA 5954
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			ACATAATA GGCTAGCTACAACGA TGATACAA 5958
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5756	GUAUCAGU A	UUAUGUAG	4673	CTACATAA GGCTAGCTACAACGA ACTGATAC	5959
5759	UCAGUAUU A	UGUAGCAU	4674	ATGCTACA GGCTAGCTACAACGA AATACTGA	5960
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5793	UUUCAGCA A	UUGAUGUC	4684	GACATCAA GGCTAGCTACAACGA TGCTGAAA	5970
5797	AGCAAUUG F	UGUCAUUU	4685	AAATGACA GGCTAGCTACAACGA CAATTGCT	5971
5799	CAAUUGAU C	UCAUUUUA	4686	TAAAATGA GGCTAGCTACAACGA ATCAATTG	5972
5802	UUGAUGUC A	AUUUUUU	4687	TAATAAAA GGCTAGCTACAACGA GACATCAA	5973
5807	GUCAUUUU A	UUAAAGAA	4688	TTCTTTAA GGCTAGCTACAACGA AAAATGAC	5974
5815	AUUAAAGA A	A CAUUGAAA	4689	TTTCAATG GGCTAGCTACAACGA TCTTTAAT	5975
5817	UAAAGAAC A	UUGAAAAA	4690	TTTTTCAA GGCTAGCTACAACGA GTTCTTTA	5976
					

Input Sequence = AF035121. Cut Site = R/Y
Arm Length = 8. Core Sequence = GGCTAGCTACAACGA
AF035121 (Homo sapiens KDR/flk-1 protein mRNA, complete cds.; Acc# AF035121; 5830 bp)

CLAIMS

- 1. A compound having Formula II: (SEQ ID NO: 5978)
- 5'-u_sa_sc_s a_sau uc<u>U</u> GAu Gag gcg aaa gcc Gaa Aag aca aB-3'

 wherein each a is 2'-O-methyl adenosine nucleotide, each g is a 2'-O-methyl guanosine nucleotide, each c is a 2'-O-methyl cytidine nucleotide, each u is a 2'-O-methyl uridine nucleotide, each A is adenosine, each G is guanosine, each s individually represents a phosphorothioate internucleotide linkage, U is 2'-deoxy-2'-C-allyl uridine, and B is an inverted deoxyabasic moiety.
 - 2. A composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier or diluent.
 - 3. A method of administering to a cell the compound of claim 1 comprising contacting said cell with the compound under conditions suitable for said administration.
 - 4. The method of claim 3, wherein said cell is a mammalian cell.
 - 5. The method of claim 3, wherein said cell is a human cell.
 - 6. The method of claim 3, wherein said administration is in the presence of a delivery reagent.
- 7. The method of claim 6, wherein said delivery reagent is a lipid.
 - 8. The method of claim 7, wherein said lipid is a cationic lipid.
 - 9. The method of claim 7, wherein said lipid is a phospholipid.
 - 10. The method of claim 6, wherein said delivery reagent is a liposome.
- 11. A method of administering to a cell the compound of claim 1 in conjunction with one or more other drug comprising contacting said cell

- with the compound and the other drug(s) under conditions suitable for said administration.
- 12. A method of inhibiting ocular angiogenesis in a subject comprising the step of contacting said subject with the compound of claim 1 under conditions suitable for said inhibition.
- 13. The method of claim 12, wherein said angiogenesis is associated with diabetic retinopathy.
- 14. The method of claim 12, wherein said angiogenesis is associated with age related diabetic retinopathy.
- 10 15. A method of cleaving RNA comprising a sequence of KDR RNA comprising contacting the compound of claim 1 with said RNA under conditions suitable for the cleavage of said RNA.
 - 16. The method of claim 15, wherein said cleavage is carried out in the presence of a divalent cation.
- 15 17. The method of claim 16, wherein said divalent cation is Mg2+.
 - 18. A method of administering to a mammal the compound of claim 1 comprising contacting said mammal with the compound under conditions suitable for said administration.
 - 19. The method of claim 18, wherein said mammal is a human.
- 20 20. The method of claim 18 wherein said administration is in the presence of a delivery reagent.
 - 21. The method of claim 18, wherein said delivery reagent is a lipid.
 - 22. The method of claim 21, wherein said lipid is a cationic lipid.
 - 23. The method of claim 21, wherein said lipid is a phospholipid.
- 25 24. The method of claim 20, wherein said delivery reagent is a liposome.

- 25. A method for treating a subject having endometriosis, comprising contacting said subject with a nucleic acid molecule that modulates the expression of VEGF, VEGFR1, and/or VEGFR2, under conditions suitable for said treatment.
- 5 26. The method of claim 25, wherein said nucleic acid molecule is an enzymatic nucleic acid molecule.
 - 27. The method of claim 25, wherein said nucleic acid molecule is an antisense nucleic acid molecule.
- The method of claim 25, wherein said nucleic acid molecule is a dsRNA nucleic acid molecule.
 - 29. The method of claim 25, wherein said nucleic acid molecule is a nucleic acid aptamer.
 - 30. The method of claim 25, wherein said nucleic acid molecule comprises a sequence having SEQ ID NO: 5977.
- The method of claim 26, wherein said enzymatic nucleic acid molecule has an endonuclease activity to cleave RNA encoded by an VEGFR1 and/or VEGFR2 gene.
 - 32. The method of claim 26, wherein said enzymatic nucleic acid molecule is in a hammerhead configuration.
- 20 33. The method of claim 26, wherein said enzymatic nucleic acid molecule is in an Inozyme configuration.
 - 34. The method of claim 26, wherein said enzymatic nucleic acid molecule is in a Zinzyme configuration.
- The method of claim 26, wherein said enzymatic nucleic acid molecule is in a DNAzyme configuration.
 - 36. The method of claim 26, wherein said enzymatic nucleic acid molecule is in a G-cleaver configuration.
 - 37. The method of claim 26, wherein said enzymatic nucleic acid molecule is in an Amberzyme configuration.

- 38. The method of claim 26, wherein said enzymatic nucleic acid molecule is an allozyme.
- 39. The method of claim 25, wherein said nucleic acid molecule is chemically synthesized.
- The method of claim 25, wherein said nucleic acid molecule comprises at least one 2'-sugar modification.
 - 41. The method of claim 25, wherein said nucleic acid molecule comprises at least one nucleic acid base modification.
- The method of claim 25, wherein said nucleic acid molecule comprises at least one phosphate backbone modification.
 - 43. The method of claim 25, wherein said subject is a human.
 - 44. A method for treating a subject having endometriosis, comprising administering to the subject a nucleic acid molecule that modulates the expression of VEGF, VEGFR1, and/or VEGFR2, under conditions suitable for said treatment.
 - The method of claim 44 wherein said administration is in the presence of a delivery reagent.
 - 46. The method of claim 45, wherein said delivery reagent is a lipid.
 - 47. The method of claim 46, wherein said lipid is a cationic lipid.
- 20 48. The method of claim 46, wherein said lipid is a phospholipid.
 - 49. The method of claim 45, wherein said delivery reagent is a liposome.
 - 50. The method of claim 44, further comprising administering one or more other drug(s).
- The method of claim 50, wherein said other drug(s) are chosen from GnRH (gonadotropin releasing hormone) agonists, Lupron Depot (Leuprolide Acetate), Synarel (naferalin acetate), Zolodex (goserelin acetate), Suprefact (buserelin acetate), Danazol, and oral contraceptives.
 - 52. A compound having Formula I: (SEQ ID NO: 5977)

5' gsasgsusugeUGAuGagg ccgaaa ggccGaaAgucugB 3'

wherein each a is 2'-O-methyl adenosine nucleotide, each g is a 2'-O-methyl guanosine nucleotide, each c is a 2'-O-methyl cytidine nucleotide, each u is a 2'-O-methyl uridine nucleotide, each A is adenosine, each G is guanosine, each s individually represents a phosphorothioate internucleotide linkage, U is 2'-deoxy-2'-C-allyl uridine, and B is an inverted deoxyabasic moiety.

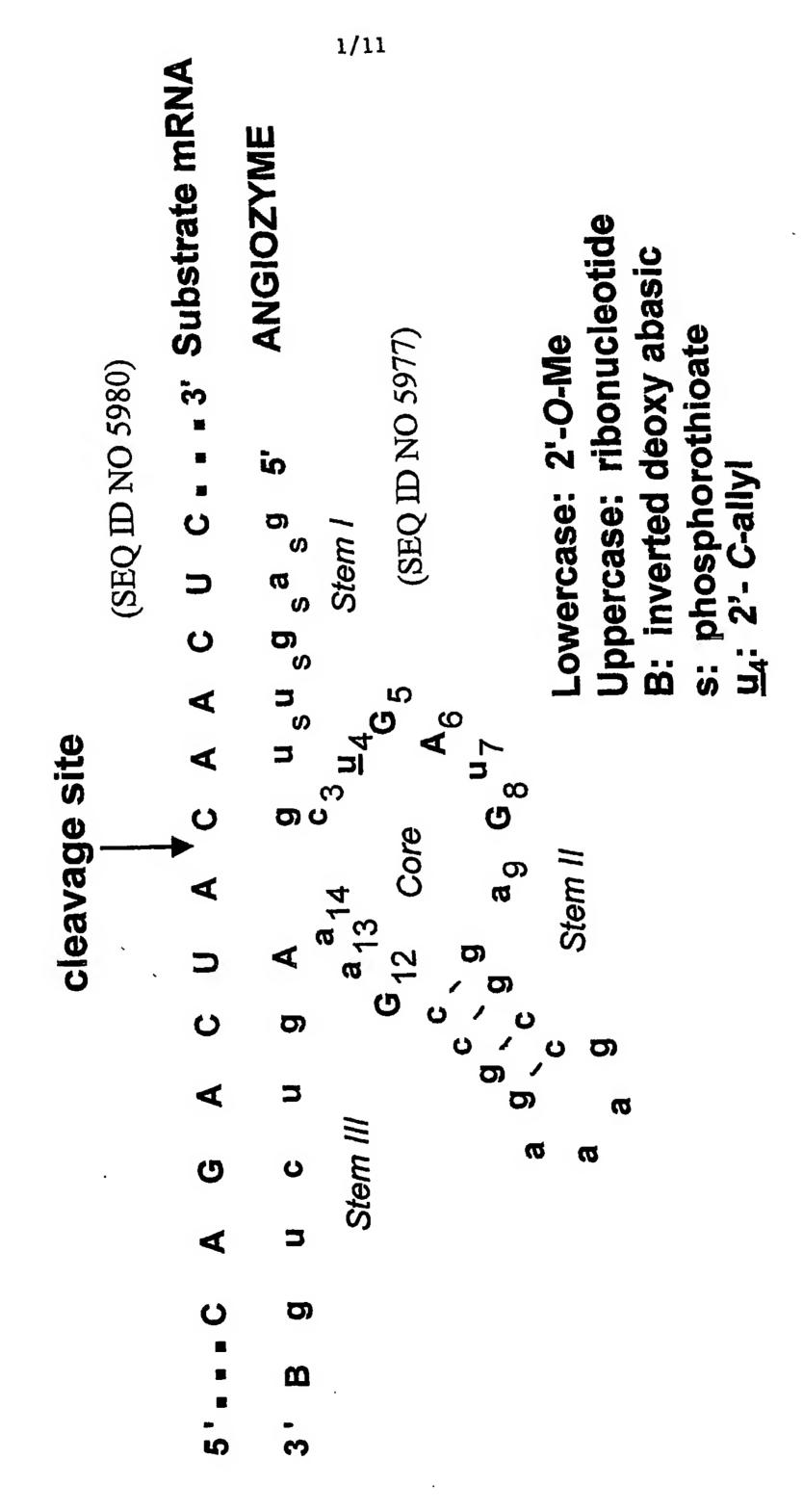
- A composition comprising a compound of claim 52 in a pharmaceutically acceptable carrier or diluent.
- 10 54. A method of administering to a cell the compound of claim 52 comprising contacting said cell with the compound under conditions suitable for said administration.
 - 55. The method of claim 54, wherein said cell is a mammalian cell.
 - 56. The method of claim 54, wherein said cell is a human cell.
- 15 57. The method of claim 54, wherein said administration is in the presence of a delivery reagent.
 - 58. The method of claim 57, wherein said delivery reagent is a lipid.
 - 59. The method of claim 58, wherein said lipid is a cationic lipid.
 - 60. The method of claim 58, wherein said lipid is a phospholipid.
- 20 61. The method of claim 57, wherein said delivery reagent is a liposome.
 - 62. A method of administering to a cell the compound of claim 52 in conjunction with a chemotherapeutic agent comprising contacting said cell with the compound and the chemotherapeutic agent under conditions suitable for said administration.
- 25 63. The method of claim 62, wherein said chemotherapeutic agent is 5-fluoro uridine.

- 64. The method of claim 62, wherein said chemotherapeutic agent is Leucovorin.
- 65. The method of claim 62, wherein said chemotherapeutic agent is chosen from Irinotecan, CAMPTOSAR®, CPT-11, Camptothecin-11, or Campto.
- 5 66. The method of claim 62, wherein said chemotherapeutic agent is Paclitaxel.
 - 67. The method of claim 62, wherein said chemotherapeutic agent is Carboplatin.
 - 68. A mammalian cell comprising the compound of claim 52...
- The mammalian cell of claim 68, wherein said mammalian cell is a human cell.
 - 70. A method of inhibiting angiogenesis in a subject, comprising the step of contacting said subject with the compound of claim 52, under conditions suitable for said inhibition.
 - 71. The method of claim 70, wherein said angiogenesis is tumor angiogenesis.
- 15 72. A method of treatment of a subject having a condition associated with an increased level of VEGF receptor comprising contacting cells of said subject with the compound of claim 52, under conditions suitable for said treatment.
- 73. The method of claim 72 further comprising the use of one or more drug therapies under conditions suitable for said treatment.
 - 74. A method of cleaving RNA comprising a sequence of VEGFR1 (flt-1), comprising contacting the compound of claim 52 with said RNA under conditions suitable for the cleavage of said RNA.
- 75. The method of claim 74, wherein said cleavage is carried out in the presence of a divalent cation.
 - 76. The method of claim 75, wherein said divalent cation is Mg2+.

- 77. The method of claim 72, wherein said condition is cancer.
- 78. The method of claim 77, wherein said cancer is breast cancer.
- 79. The method of claim 77, wherein said cancer is lung cancer.
- 80. The method of claim 77, wherein said cancer is colorectal cancer.
- 5 81. The method of claim 77, wherein said cancer is renal cancer.
 - 82. The method of claim 77, wherein said cancer is melanoma.
 - 83. The method of claim 77, wherein said cancer is pancreatic cancer.
 - 84. The method of claim 79, wherein said lung cancer is non-small cell lung carcinoma.
- 10 85. The method of claim 81, wherein said renal cancer is renal cell carcinoma.
 - 86. The method of claim 73, wherein said other therapy is 5-fluoro uridine.
 - 87. The method of claim 73, wherein said other therapy is Leucovorin.
 - 88. The method of claim 73, wherein said other therapy is Irinotecan, CAMPTOSAR®, CPT-11, Camptothecin-11, or Campto.
- 15 89. The method of claim 73, wherein said other therapy is Paclitaxel.
 - 90. The method of claim 73, wherein said other therapy is Carboplatin.
 - 91. A method of administering to a mammal the compound of claim 52 comprising contacting said mammal with the compound under conditions suitable for said administration.
- 20 92. The method of claim 91, wherein said mammal is a human.
 - 93. The method of claim 91, wherein said administration is in the presence of a delivery reagent.
 - 94. The method of claim 93, wherein said delivery reagent is a lipid.

- 95. The method of claim 94, wherein said lipid is a cationic lipid.
- 96. The method of claim 94, wherein said lipid is a phospholipid.
- 97. The method of claim 93, wherein said delivery reagent is a liposome.
- 98. A method of administering to a mammal the compound of claim 52 in conjunction with a chemotherapeutic agent comprising contacting said mammal with the compound and the chemotherapeutic agent under conditions suitable for said administration.
 - 99. The method of claim 98, wherein said chemotherapeutic agent is 5-fluoro uridine.
- 10 100. The method of claim 98, wherein said chemotherapeutic agent is Leucovorin.
 - The method of claim 98, wherein said chemotherapeutic agent is Irinotecan, CAMPTOSAR®, CPT-11, Camptothecin-11, or Campto.
 - 102. The method of claim 98, wherein said chemotherapeutic agent is Paclitaxel.
- 15 103. The method of claim 98, wherein said chemotherapeutic agent is Carboplatin.

Figure 1: Anti-Flt-1 Ribozyme: ANGIOZYME



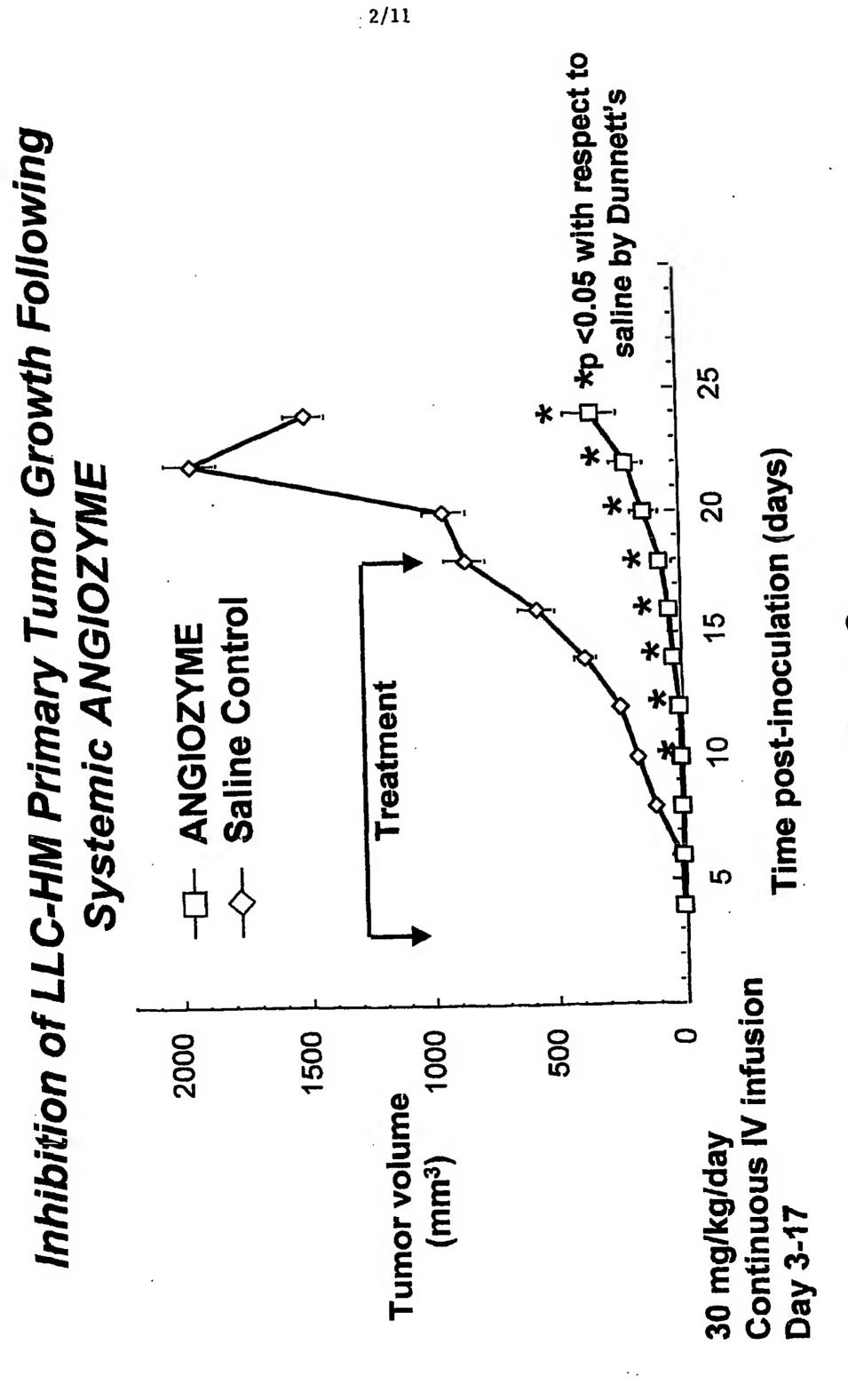
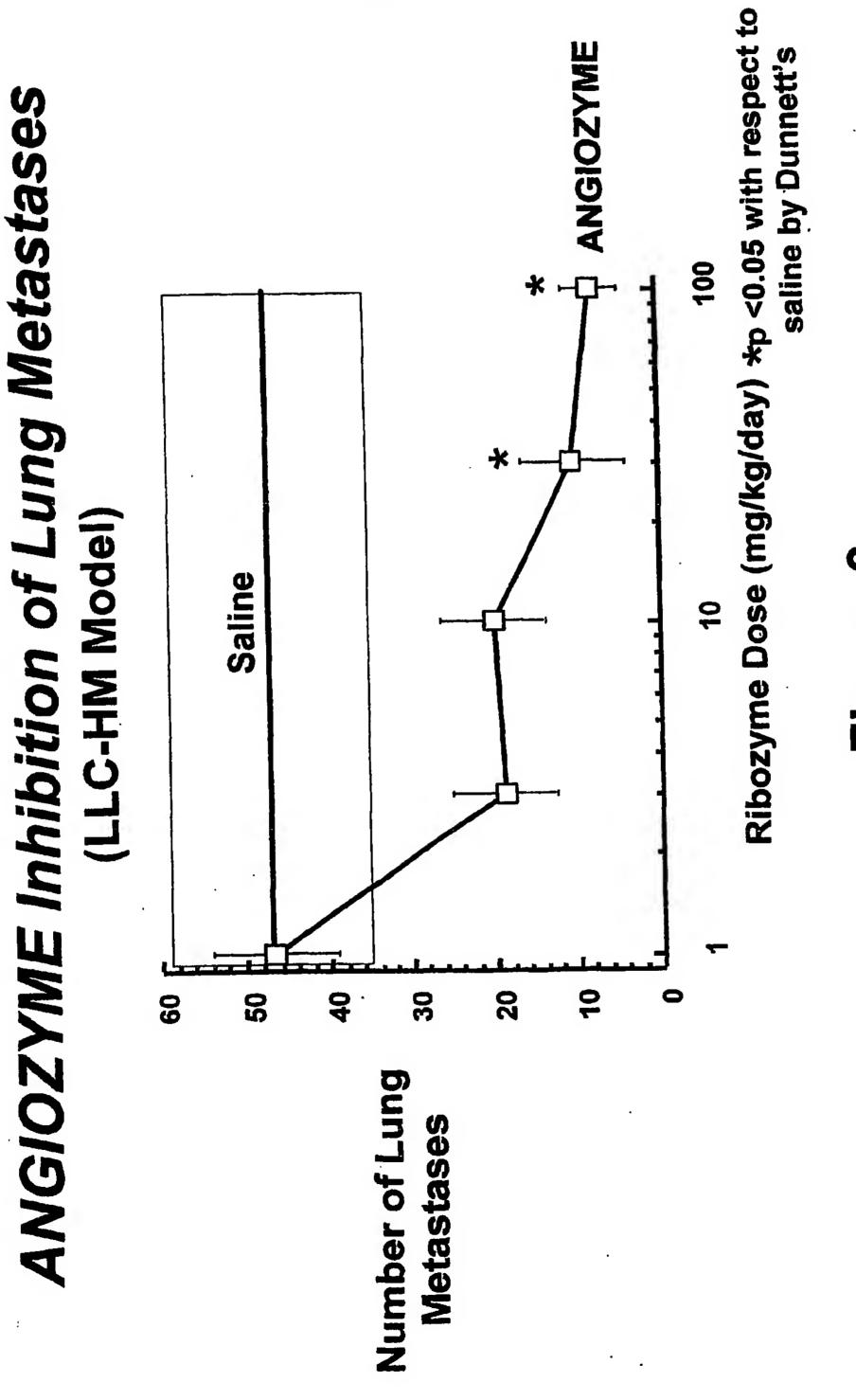


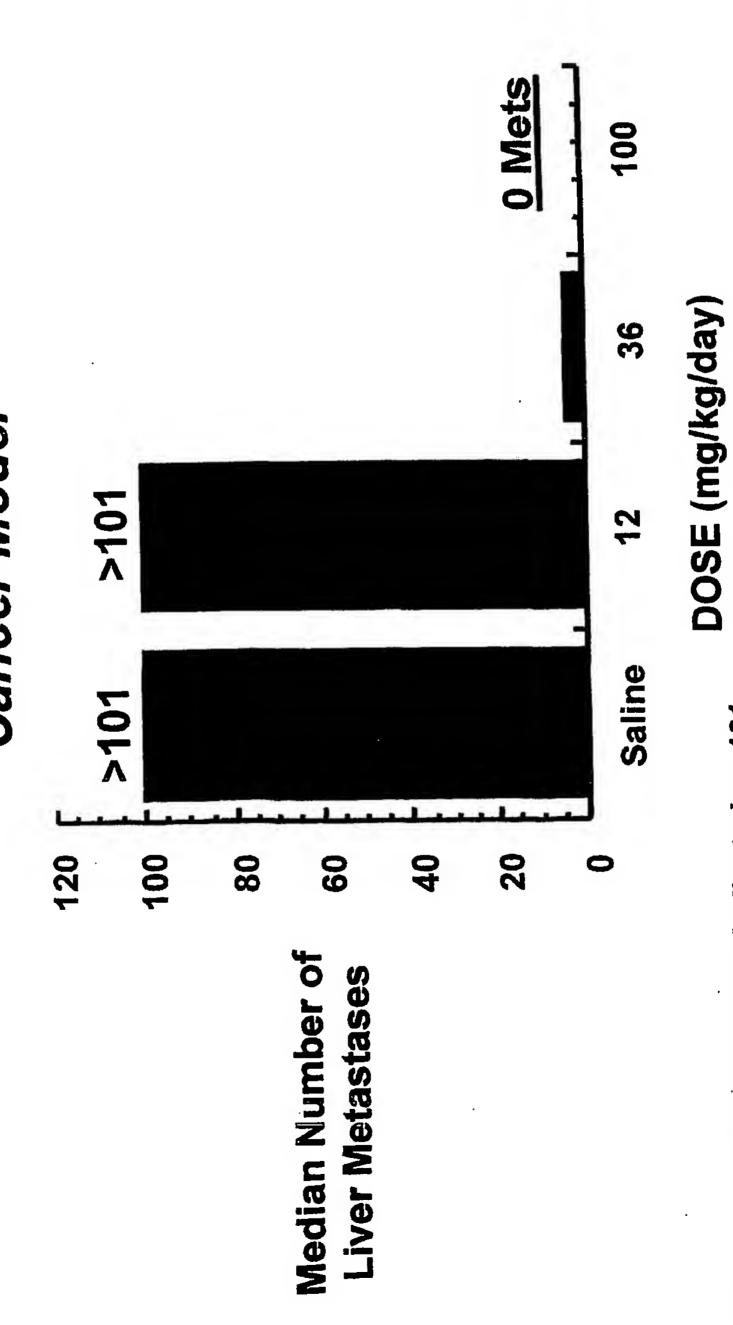
Figure 2



3/11

Figure 3

on Liver Metastases in a Colorectal Sancer Model Effect of ANGIOZYME



Note: > 100 metastases are indicated as 101.

Figure 4

Figure 5: Plasma concentration profile of ANGIOZYME after a single subcutaneous dose of 10, 30, 100 or 300 mg/m²

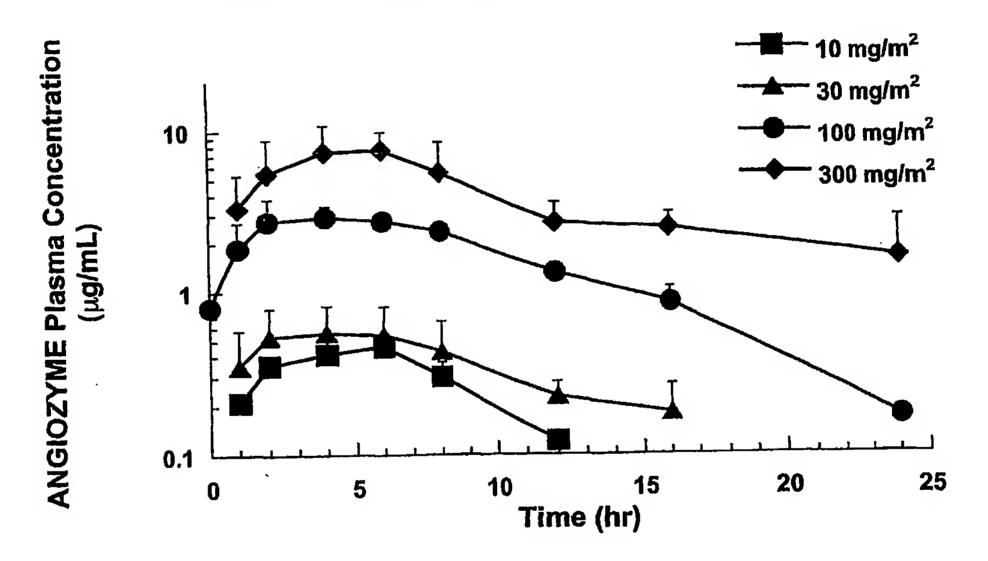


Figure 6: Examples of Nuclease Stable Ribozyme Motifs

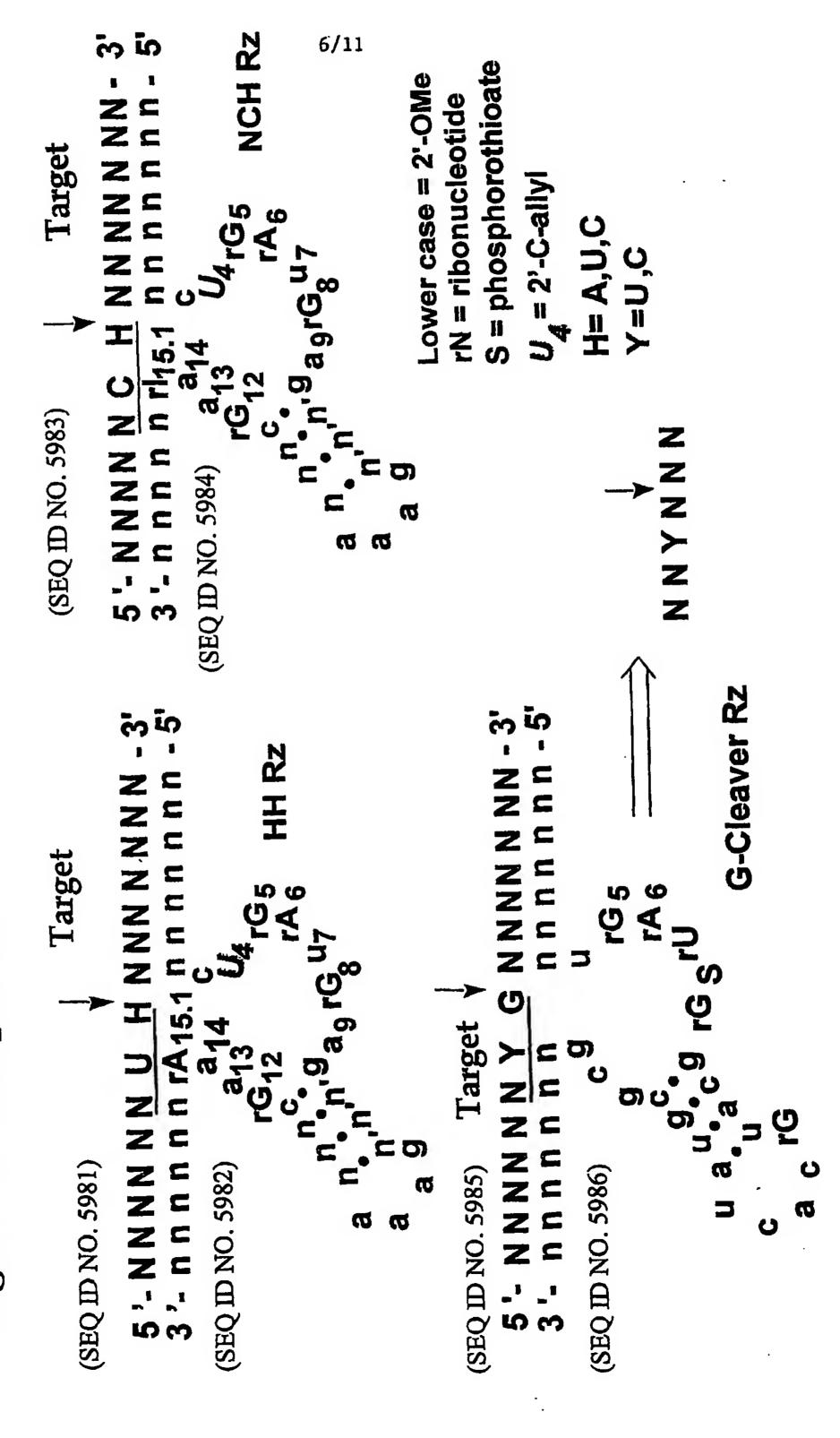
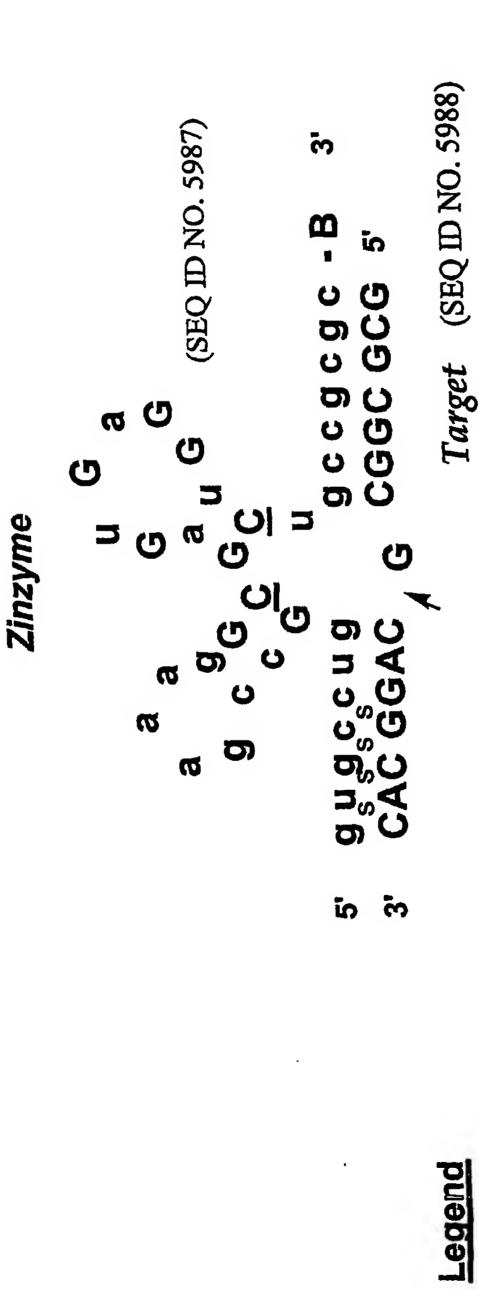


Figure 7: Stabilized Zinzyme Ribozyme Motif



Uppercase: indicates natural ribo residues

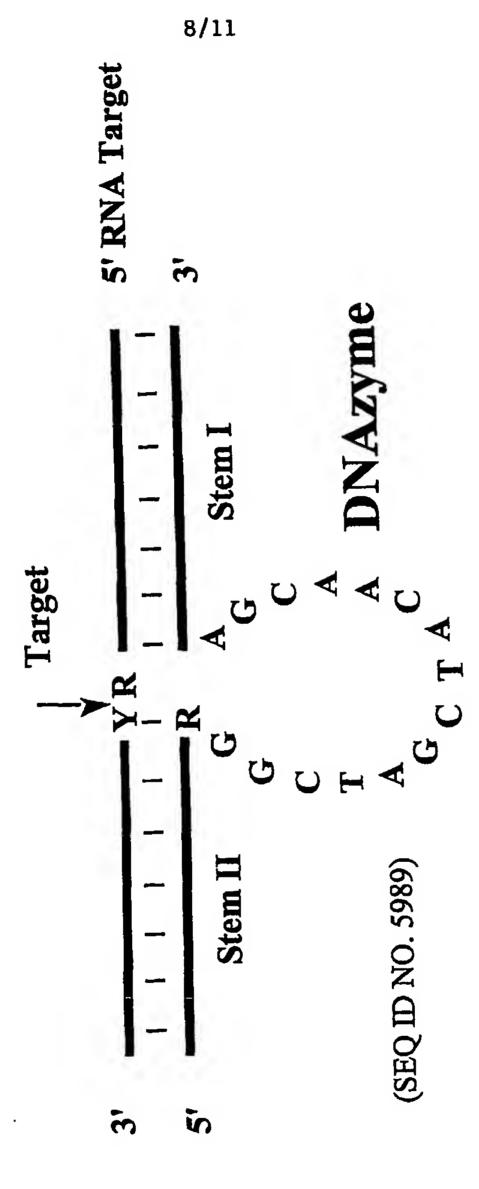
C: indicates 2'-deoxy-2'-amino Cytidine

Lowercase: 2'-0-methyl

S: phosphorothioate/phosphorodithioate linkage

B: 3'-3' abasic moiety

Figure 8: DNAzyme Motif



Legend
Y = U or C
R = A or G

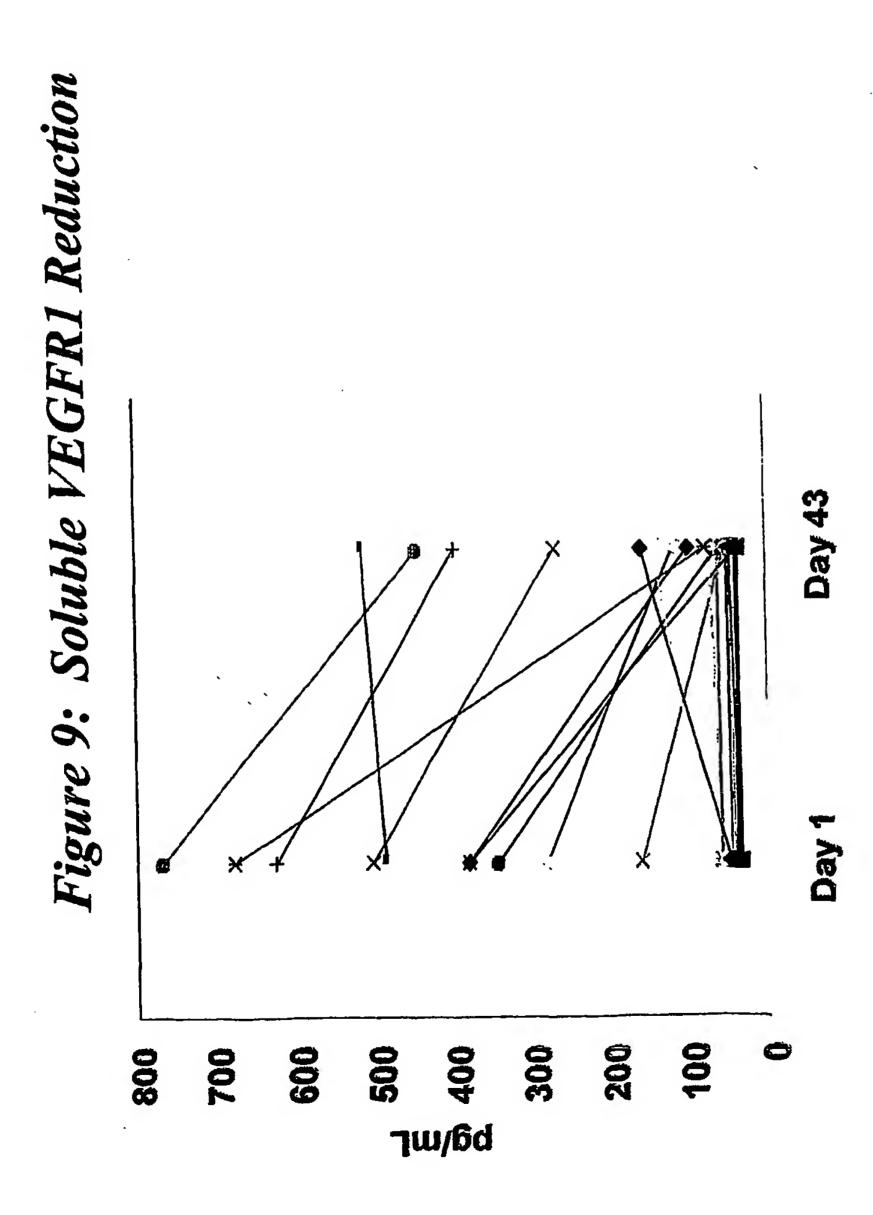
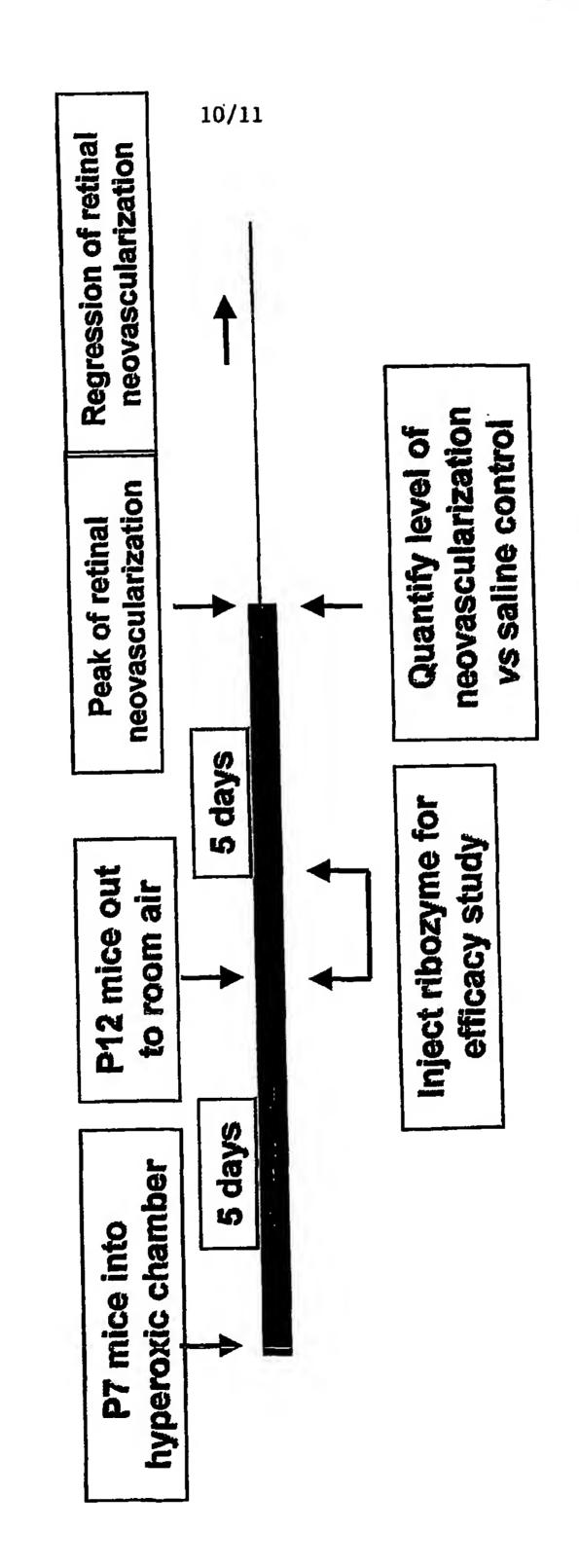
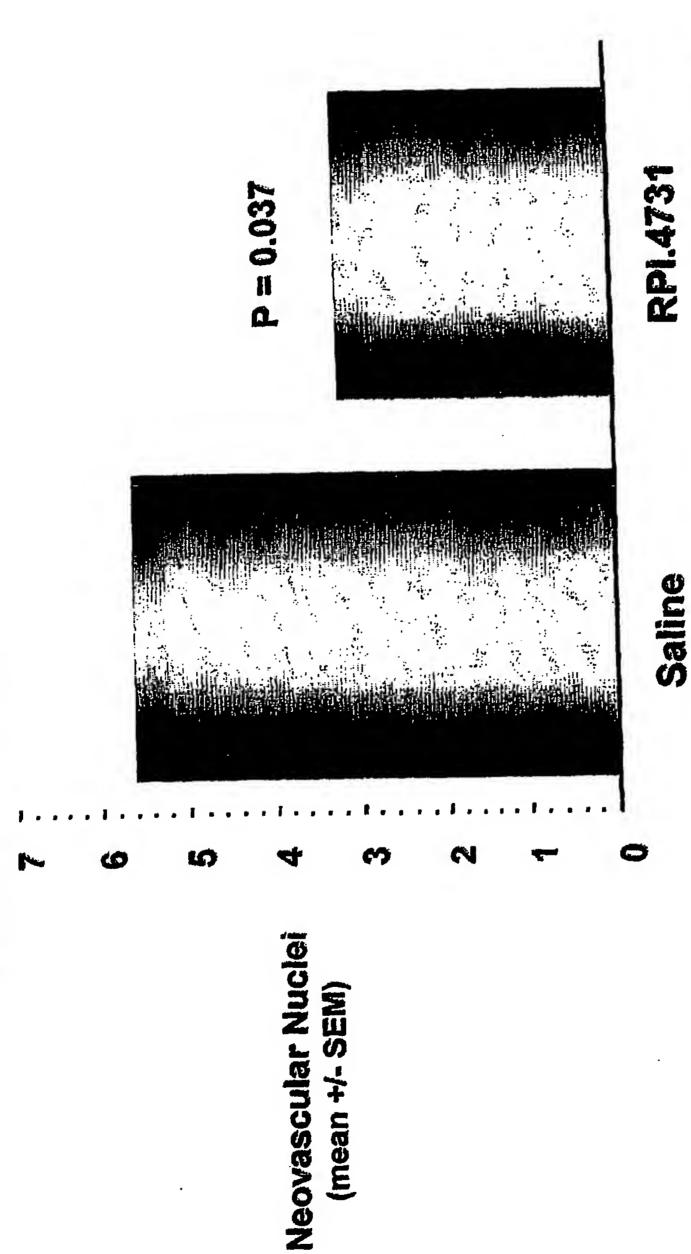


Figure 10: Mouse Model of Proliferative Retinopathy



12 hr after exposure to room air Note: Peak VEGF levels noted





SEQ ID NO: 5978 Results: ~40% decrease in retinal neovascularizati following two intraocular injections of RPI.473'

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